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PROPHYLAXIS AND TREATMENT OF CYANIDE INTOXICATION
Cyanide - Mechanism of Prophylaxis

Annual Report

James L. Way

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see SUMMARY section of report

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FOREWORD

In conducting the research described in this report, the investigator(s)
adhered to the "Guide for the Care and Use of Laboratory Animals,"
prepared by the Committee on Care and Use of Laboratory Animals of
the Institute of Laboratory Animal Resources, National Research Council
(DHEW Publication No. (NIH) 78-23, Revised 1978).

CONTENTS

SUMMARY

One treatment for cyanide poisoning involves administration of the combination sodium thiosulfate and sodium nitrate (1). Thiosulfate is believed to exert its antidotal effects by serving as a sulfur donor, thereby increasing the rate of rhodanase catalyzed biotransformation of cyanide to thiocyanate. To understand better the action of thiosulfate on cyanide toxicity, a study was made of the pharmacokinetics of cyanide distribution and metabolism in the absence and presence of thiosulfate. Fasted mongrel dogs were anesthetized with sodium pentobarbital and both jugular veins were catheterized. Thiosulfate, when present, was maintained at a constant concentration in the plasma by administration of a bolus followed by a constant rate infusion of a solution of thiosulfate into one of the catheters. The concentrations of both cyanide and thiocyanate were determined using a potentiometric method in combination with microdiffusion procedures (2,3). Thiosulfate did not alter the kinetics of distribution or elimination of thiocyanate. A pharmacokinetic model of thiocyanate distribution and elimination was developed based on thiocyanate plasma concentration that were measured after i.v. administration. Thiosulfate was shown to not affect the model parameters for thiocyanate . The thiocyanate model was coupled with a model for cyanide and the blood levels of cyanide and thiocyanate measured after administration of cyanide was used to determine values for the cyanide model parameters. This kinetic analysis showed that: 1) thiosulfate increased the rate of conversion of cyanide to thiocyanate by a factor of 36.5 and 2) the apparent volume of distribution of cyanide was reduced by thiosulfate. The mechanism of thiosulfate protection appeared to be due to an initial burst of formation of thiocyanate formation and thus limited the amount of cyanide distributed peripherally.

Studies involving cyanide in combination with various antidotal regimens indicated that sodium thiosulfate, interfered with the potentiometric determination of cyanide. The basis for this interference is ascribed to an enhanced biotransformation of thiosulfate in the presence of blood. A microdiffusion technique, coupled with a silver/sulfide ion specific electrode caused falsely elevated cyanide levels when samples contained thiosulfate. The contaminant causing the falsely elevated cyanide level is believed to be sulfide anion. This sulfide contaminant can be removed by oxidation with hydrogen peroxide, and the excess hydrogen peroxide subsequently can be eliminated with sodium sulfite. The toxicologic implications of the potentiometric determination of cyanide in the presence of sodium thiosulfate are discussed.

A review on cyanide is enclosed. This review is published in Trends in Pharmacological Science.

PART I

EFFECTS OF THIOSULFATE ON CYANIDE PHARMACOKINETICS IN DOGS

PART I

EFFECTS OF THIOSULFATE ON CYANIDE PHARMACOKINETICS IN DOGS

INTRODUCTION

The major toxic effect of cyanide, inhibition of cytochrome oxidase, occurs almost entirely intracellularly. Rhodanese mediated detoxication of cyanide to thiocyanate also takes place intracellularly, since rhodanese is a mitochondrially bound enzyme. A variety of treatments for cyanide poisoning have been advocated, including the administration of sodium thiosulfate (Chen and Rose, 1952). While it is believed that thiosulfate acts by supplying sulfur to rhodanese, this action is relatively limited, because rhodanese is located in the mitochondria. The detoxication potential of this enzyme is relatively low in vivo when compared to the higher enzymatic activity observed in vitro when the enzyme is lysed and released from the mitochondria. This is probably due to the low permeability of the cell and mitochondrial membranes to the thiosulfate anion (Sorbo, 1975; Koj, 1979). [Crompton et al. (1974) showed that thiosulfate transport to mitochondria is catalyzed by a dicarboxylate carrier.] The pharmacokinetics of cyanide and thiocyanate in the presence and absence of sodium thiosulfate, as shown in this study, indicates that the mechanism of thiosulfate protection may not be wholly rhodanese-mediated.

It was necessary to characterize the pharmacokinetics of thiocyanate in order to describe more completely the pharmacokinetics of cyanide. Auto-radiographs from whole body sections of mice which received radiolabeled

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thiocyanate showed that thiocyanate is not uniformly distributed, but that it concentrates in certain organs, such as the stomach (Clemedson et al., 1960). For this reason, the conclusion based on earlier studies, that thiocyanate space equalled the extracellular fluid volume, may not be valid (Crandall and Anderson, 1934; Eder, 1951; Carlson et al., 1979). Therefore, the extracellular fluid volume may not be an accurate estimate of the thiocyanate distribution space. The apparent volume of distribution of thiocyanate has been determined by several investigators; thiocyanate space expressed as a percentage of body weight varied from 25% to 52% (Eder, 1951; Carlson et al., 1979; Crandall and Anderson, 1934; Elkinton and Taffel, 1942). No detailed study of the pharmacokinetics of thiocyanate could be found.

Pharmacokinetic analysis of cyanide distribution and metabolism is essential in order to gain more insight into the disposition of cyanide in vivo. Such knowledge may lead to a better understanding of the mechanism of antidotal activity and it may be useful in contributing to a more efficacious treatment and assessment of cyanide toxicity in humans. Earlier studies revealed that approximately 10% to 20% of the radioactivity administered as cyanide was found in the urine as thiocyanate (Burrows et al., 1976; Christel et al., 1977) whereas, in animals treated with thiosulfate, up to 60% of a dose of cyanide was excreted as urinary thiocyanate (Smith et al., 1940).

Preliminary results from a study of the pharmacokinetics of cyanide in dogs indicate a striking difference between cyanide blood levels in control and thiosulfate-treated animals within 5 minutes after cyanide was administered intravenously. Dogs receiving cyanide alone exhibited apnea which was not apparent in thiosulfate-treated dogs; therefore, it appeared reasonable to assume that, in thiosulfate-treated animals, the enhanced respiration may have increased the pulmonary excretion of cyanide. The studies were expanded to include the

measurement of the respiratory excretion of cyanide in control and thiosulfate-treated animals. Furthermore, the evaluation of cyanide antagonists should include a study of the effects of the antidote on the pharmacokinetics of cyanide. This study describes a kinetic analysis of whole blood cyanide concentration as well as plasma thiocyanate concentrations after the administration of a sublethal dose of cyanide to control dogs, and to dogs treated with thiosulfate.

MATERIALS AND METHODS

Materials

Potassium cyanide and sodium dihydrogen phosphate were products of Fisher Scientific Company (Fairlawn, NJ). Sodium thiosulfate, sodium hydroxide, sodium cyanide, sodium acetate, glacial acetic acid, and potassium permangenate were obtained from J. T. Baker Chemicals (Phillipsburg, NJ). Sodium dodecyl sulfate (SDS) was purchased from Sigma Chemical Company (St. Louis, MO). Silver potassium cyanide was a product of K and K Laboratories, Inc. (Plainview, NY). Ethylenediamine was obtained from Eastman Organic Chemicals (Rochester, NY) and redistilled before use. Sodium heparin and sodium pentobarbital were from Abbott Laboratories (North Chicago, IL). All other chemicals used were of the highest grade of purity commercially available.

Methods

Cyanide and Thiocyanate Determination

Cyanide and thiocyanate were subjected to microdiffusion and analyzed potentiometrically (Feldstein and Klendshoj, 1954; Blaedel, 1971). The microdiffusion was conducted in porcelain Conway diffusion cells (A. H. Thomas Company, Philadelphia. '' A ml aliquot of 1.0 M sodium acetate buffer,

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pH 5.2, and 0.5 ml of a 0.1 mM solution of SDS was added to the outer well of the diffusion cell, and 2.0 ml of 0.1 N sodium hydroxide was added to the inner well. When thiocyanate was determined, 0.5 ml of 0.1 M potassium permanganate was added to the outer well to oxidize the thiocyanate to cyanide. A sample of whole blood, plasma, or standard solution was added to the outer well of the microdiffuson cell, and the cell was sealed immediately with a lubricated ground glass plate. The diffusion cells were inclined 3° from horizontal and rotated six times during a three hour diffusion at room temperature.

Cyanide was determined potentiometrically using a silver/sulfide ion specific electrode with a model 701 A Orion digital pH meter (Orion Research, Inc.) that contained 10 μl of a silver potassium cyanide indicator-buffer solution (17.69 g Na_2HPO_4, 5.5 ml 10 M NaOH, 10 mg silver potassium cyanide indicator, and 3.3 ml ethylenediamine/100 ml). The potential in millivolts was determined while the sample was gently agitated. Samples of cyanide concentration were determined and compared with a series of cyanide standards which were analyzed daily. Thiocyanate concentration was determined from the difference between an oxidized and non-oxidized sample.

Cyanide and Thiocyanate Pharmacokinetics

Thiocyanate

Fasted mongrel dogs were injected intraveneously either with a loading dose solution (0.0963 gm/kg) of sodium thiosulfate or an equal volume of saline. Fifteen minutes later, an intravenous bolus of sodium thiocyanate was administered (2 mg/kg). Heparinized blood samples were withdrawn over a twelve-hour period and the plasma was then separated and analyzed for thiocyanate concentration. At least five animals were used for each experiment.

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Cyanide

Fasted animals were anesthetized with sodium pentobarbital and the two jugular veins were catheterized percutaneously (Venocath-16 catheters, Abbott Laboratories). One catheter was used for the infusion of saline or sodium thiosulfate and other for the administraiton of cyanide and the withdrawal of blood samples. Control animals received a constant infusion of isotonic saline (1.57 ml/min) for thirty minutes prior to injection of cyanide. Thiosulfate-treated animals received a loaidng dose of sodium thiosulfate (0.0963 gm/kg) followed by an infusion of thiosulfate solution (11.4 μmoles/kg/min) to achieve a steady state level of 2 μmoles/ml. Thirty minutes after starting the infusion, sodium cyanide was injected intravenously. Heparinized blood samples were withdrawn at various times and analyzed for cyanide and thiocyanate. General anesthesia was maintained throughout the study by intravenous injection of small amounts of sodium pentobarbital. The catheter used for cyanide administration and blood sampling was rinsed with heparinized saline after each use to minimize clotting within the catheter.

Pulmonary Excretion Study

Dogs were prepared as described for the determination of the pharmacokinetics of cyanide. In addition, endotrachael tubes were inserted and checked for a tight trachael seal. The trachael tube was connected to a spirometer to measure expired air volume. Air exiting the spirometer bubbled through a solution of 0.1 N NaOH to trap cyanide. The collection flasks were arranged as shown in Figure 6.1 to collect samples at various time intervals. The sodium hydroxide solution was then analyzed for cyanide content. When known amounts of cyanide were passed through the collection system, 85% of the cyanide was recovered in the sodium hydroxide solution. Other measurements made included respiration rate, and venous blood gases for the first five

minutes after injection of sodium cyanide. Blood gases were analyzed on an IL Micro 13 (International Laboratories, Newton, MA).

Data Analysis

Compartmental pharmacokinetic models were used; model-based equations were fit to the data using the NONLIN computer program (Metzler et al., 1974). Averaged data were used and each mean was weighted by the reciprocal of its standard error. Also, the blood cyanide function in animals treated with thiosulfate was increased by a factor of 10 in order to give equal weighting to both the cyanide and thiocyanate functions.

The plasma concentration of thiocyanate appeared to be a biexponential function of time (Fig. 6.2) and a standard two-compartment model was therefore used for this compound (Fig. 6.3). The following equations were fit to the data to obtain estimates of K_{12}, K_{21}, K_{10}, and V_1:

$$\frac{dC_c}{dt} = K_{12}C_p - (K_{12} + K_{10})C_c \tag{1}$$

$$\frac{dC_p}{dt} = K_{21}(C_c - C_p) \tag{2}$$

C_c and C_p refer to the concentration of thiocyanate in compartments 1 and 2, respectively. An estimate for V_2 was obtained from:

$$V_2 = K_{12}V_1/K_{21} \tag{3}$$

The disappearance of cyanide after its i.v. administration appeared to be monoexponential (Fig. 6) and a one-compartment model appeared to be appropriate. The model for cyanide was coupled reversibly to the thiocyanate model (Fig. 6.7), since thiocyanate can revert to cyanide (Lang, 1933).

Model parameters for thiocyanate ($V_1, K_{12}, K_{21}, K_{10}$) were fixed at the values that were determined after administration of thiocyanate. The following equations were fit simultaneously to whole blood concentrations of cyanide (C_{CN}) and plasma concentrations of thiocyanate (C_c) to obtain estimates of K_{13}, K_{31}, K_{30}, and V_3:

$$\frac{dC_{CN}}{dt} = \frac{K_{21}CON(4)C_c}{V_1} - (K_{12} + K_{10})C_{CN} \tag{4}$$

$$\frac{dC_c}{dt} = \frac{K_{12}V_1 C_{CN}}{CON(4)} + CON(2)C_p - [CON(2) + K_{21} + CON(1)]C_c \tag{5}$$

$$\frac{dC_p}{dt} = CON(3)(C_c - C_p) \tag{6}$$

The fit of equations 4-6 to data that were collected from thiosulfate treated animals was not satisfactory. The NONLIN program converged on a relatively small volume for V_3, that was approximately equal to the blood volume. It appeared that in the antidote treated animals, cyanide was converted to thiocyanate primarily in the blood, prior to its distribution. A model consistent with this possibility was developed using two compartments for cyanide; a small central compartment where thiocyanate was formed from cyanide, and a peripheral compartment that also allowed for elimination of cyanide (Fig. 6.4). In addition to equations 5 and 6, the following equations were used for this model:

$$\frac{dC_{pCN}}{dt} = K_{31}C_{cCN} - (K_{31} + K_{10})C_{pCN} \tag{7}$$

$$\frac{dC_{cCN}}{dt} = \frac{K_{21}CON(4)C_c}{V_2} + C_{pCN}K_{13} - (K_{12} + K_{13})C_{cCN} \tag{8}$$

C_{cCN} and C_{pCN} refer to the concentrations of thiocyanate in compartments 3 and 4, respectively. As with the control, model parameters for
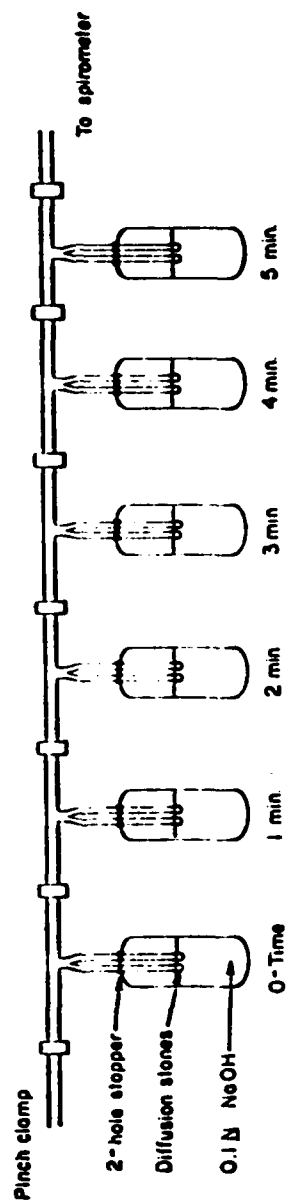
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Fig. 6.1. Collection Flasks for Expired Cyanide.

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thiocyanate were fixed at the values determined from data collected after thiocyanate administration.

RESULTS

Thiocyanate

Plasma concentrations of thiocyanate after rapid i.v. administration of the compound to control and thiosulfate treated dogs is shown in Fig. 6.2. The agreement between the 2 compartment model for thiocyanate (Fig. 6.3) and this data is illustrated in Fig. 6.4 and 6.5. The correlation coefficients were 0.997 and 0.994 for the control and thiosulfate treated animals, respectively. Thiosulfate model parameters are presented in Table 6.1; the presence of thiosulfate did not significantly alter their magnitude. The total body clearance of thiocyanate ($K_{10} \cdot V_1$) was 11.4 and 13.3 ml/hr/kg in control and thiosulfate treated animals. The corresponding biological half-lives ($t_{1/2}$) , were 29 and 25 hours, respectively, and the apparent steady-state volumes of distribution ($V_1 + V_2$) were 470 and 472 ml/kg. Of this total volume, 86% was associated with the central compartment.

Cyanide

The disappearance of cyanide from the blood and the appearance of thiocyanate in the plasma are shown in Figs. 6.6 and 6.7 for control and thiosulfate treated dogs. Blood concentrations of cyanide appeared to decrease linearly on similogarithmic coordinates (Fig. 6). Both plots appeared to be parallel with the concentrations in thiosulfate treated animals averaging about 65% of those of control animals. Plasma concentrations of thiocyanate increased to a common plateau (Fig. 7). The plateau was reached in thiosulfate treated animals before the first sample was taken (5 minutes) while 2 hours were required in control animals. The fit of equations based on the
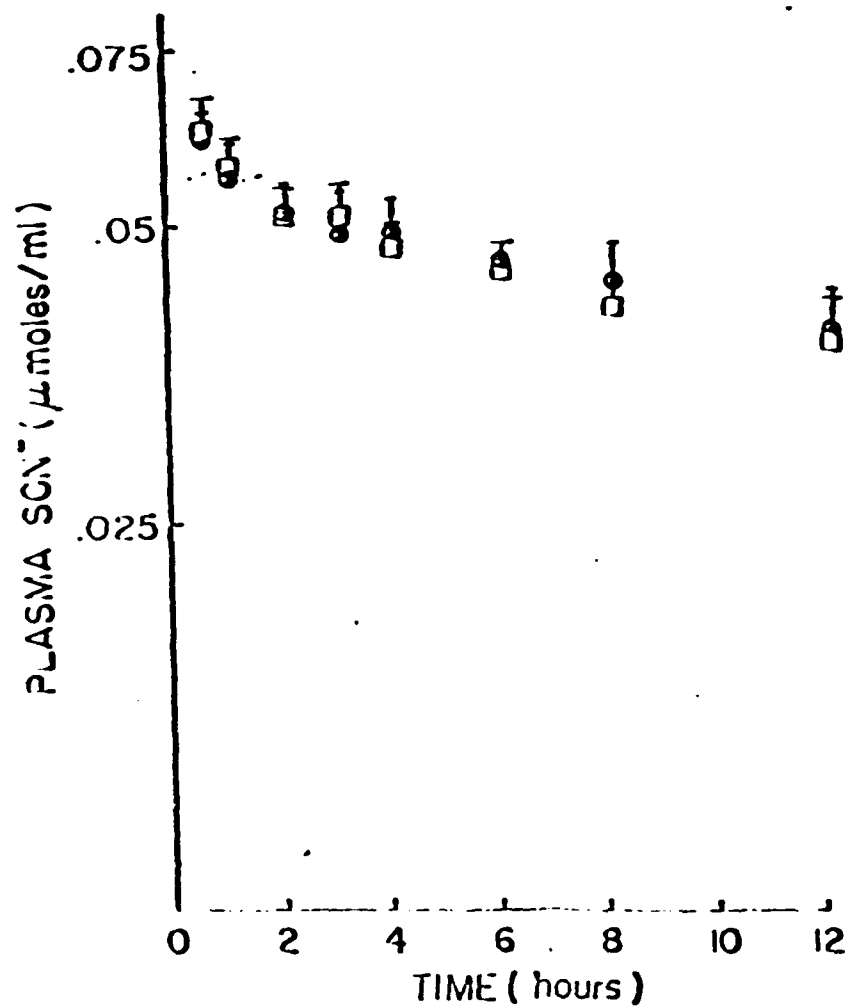
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Fig. 6.2. Plasma Thiocyanate Concentration Vs.
Time Showing Mean ± SEM.
□ Control
● Thiosulfate treated
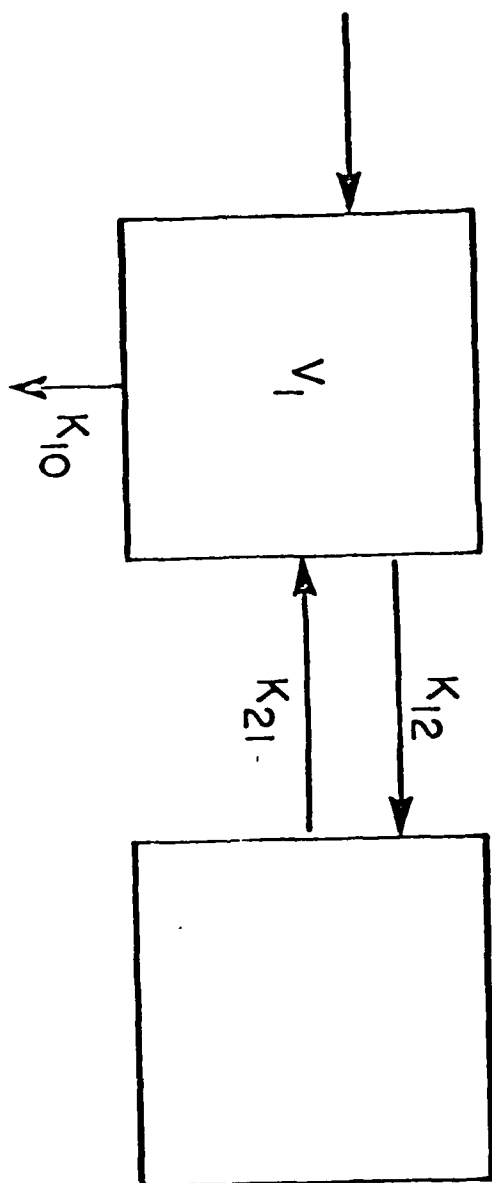Each point represents the mean ± SEM
of 5 dogs.

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Fig. 6.3. Thiocyanate Pharmacokinetic Model of Distribution and Elimination.

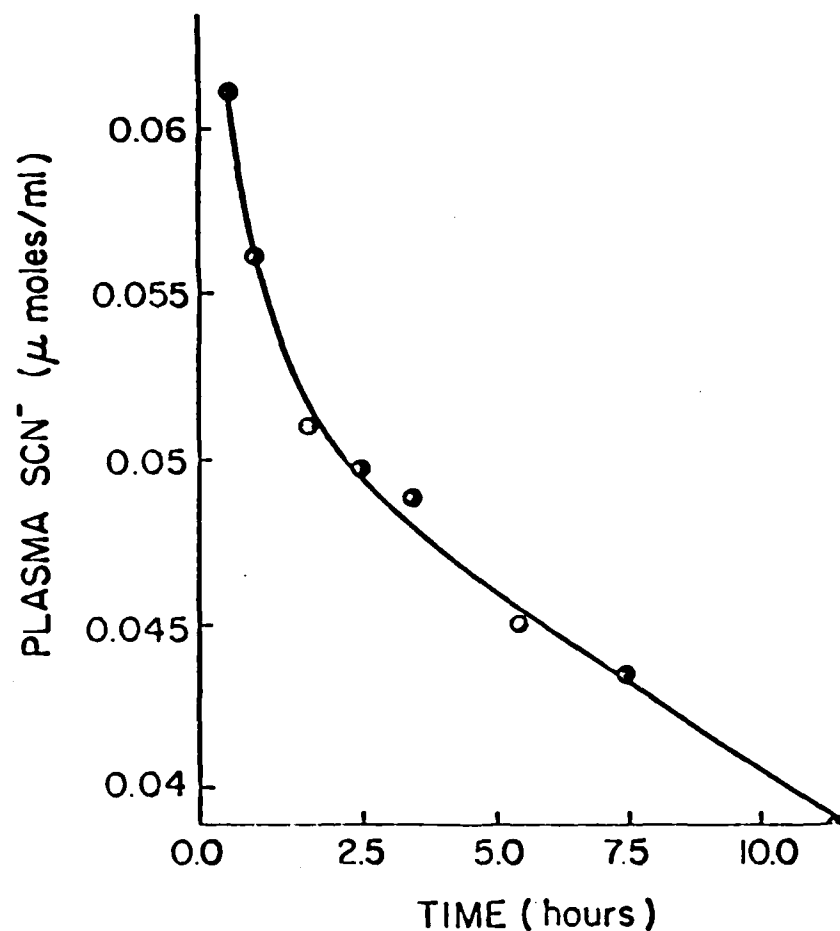Fig. 6.4. Thiocyanate Model Predicted
Distribution and Elimination for
Control Dogs.
—— Model predicted using Eqs. 1
and 2 and parameters listed in
Table 6.1.
● Experimentally determined

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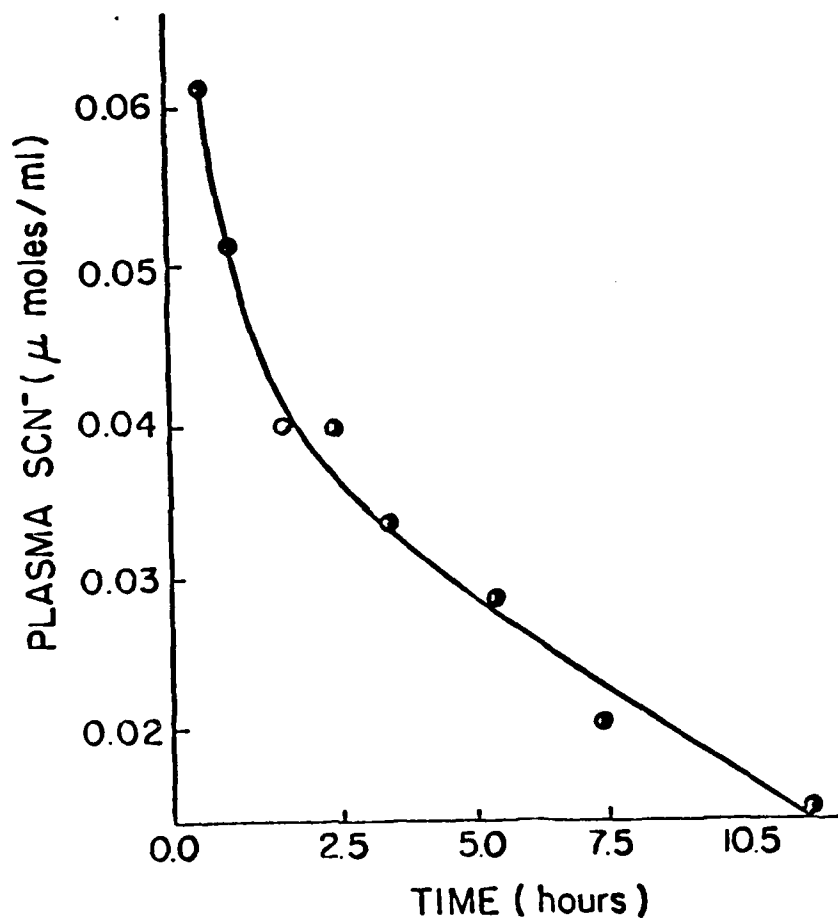
Fig. 6.5. Thiocyanate Model Predicted
Distribution and Elimination for
Thiosulfate Pretreated Dogs.
—— Model predicted using Eqs. 1
and 2 and parameters listed in
Table 6.1.
● Experimentally determined

14
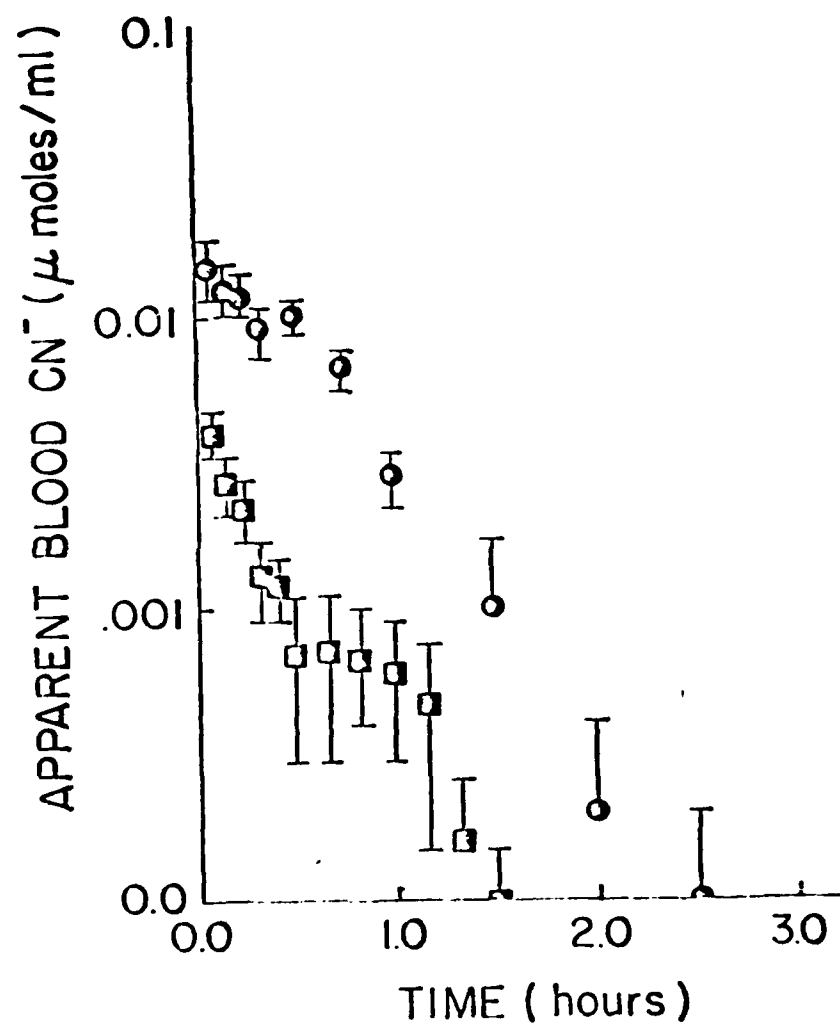
Fig. 6.6 Blood Cyanide Concentration Vs.
Time Showing the Mean Values and
the SEM.
● Control
■ Thiosulfate pretreated
Each point represents the mean
value of. 6 dogs.

15
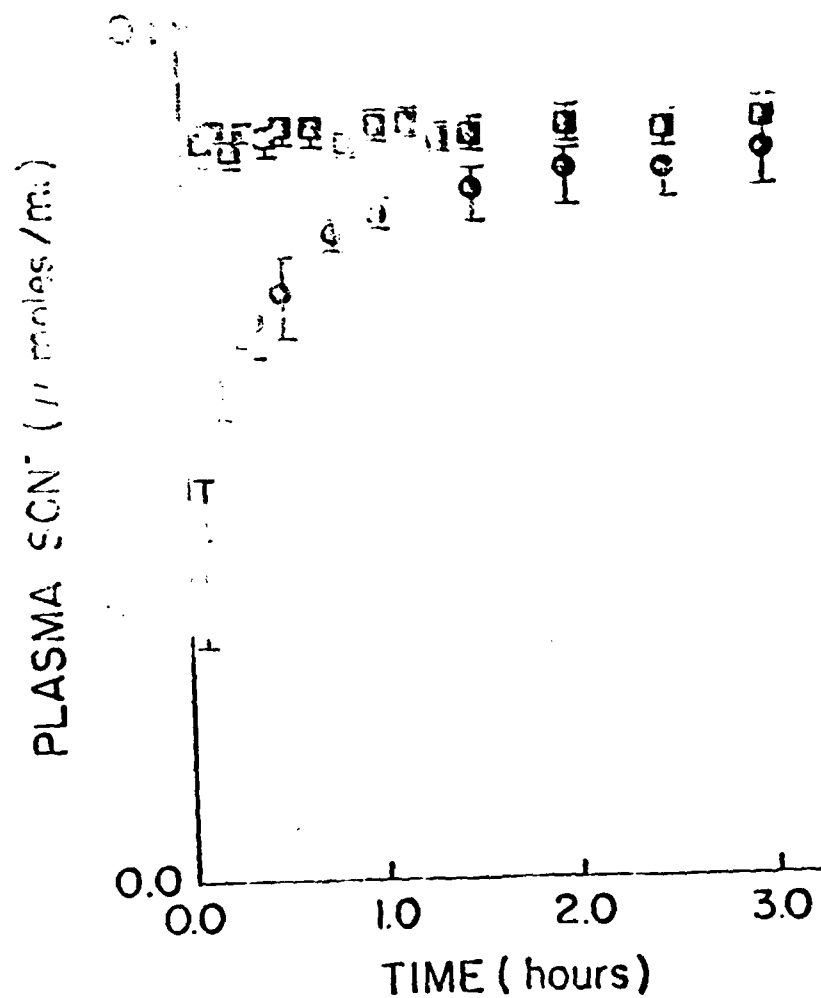
Fig. 6.7. Plasma Thiocyanate Concentration
Vs. Time Showing the Mean Values
and the SEM.
● Control
■ Thiosulfate pretreated
Each point represents the mean
value of 6 dogs.

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Fig. 6.8. Cyanide Pharmacokinetic Model of Distribution and Elimination (Control Dogs).

Con(1) refers to V_1 listed in Table 6.1.
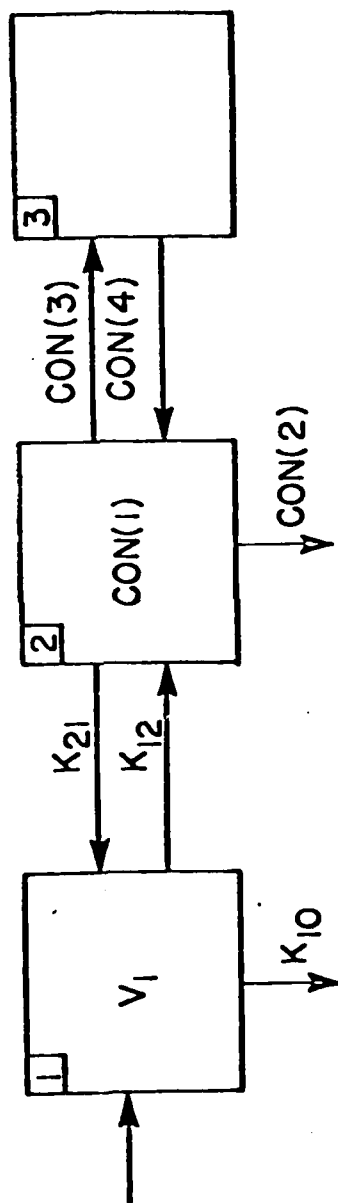Con(2) refers to K_{10}^1 listed in Table 6.1.
Con(3) refers to K_{12}^1 listed in Table 6.1.
Con(4) refers to K_{21}^1 listed in Table 6.1.

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model shown to cyanide and thiocyanate concentrations in control animals is shown in Figs. 6.10 and 6.11; correlation coefficients for the two plots were 0.983 and 0.988, respectively, and the sums of squares were 1.18×10^{-4} and 0.812×10^{-4} (Table 6.1). Least squares estimats of the model parameters are given in Table 6.2. The fit of the model illustrated in Fig. 6.9 to cyanide and thiocyanate concentrations in thiosulfate treated animals is shown in Figs. 6.12 and 6.13. Correlation coefficients for the fits were 1.00 and 0.979 for cyanide and thiocyanate, respectively, and sums of squares were 3.4×10^{-4} and 1.1×10^{-4} (Table 6.2). Estimates of the model parameters are presented in Table 6.3.

The apparent steady-state volumes of distribution of cyanide, V_1 in the control model and $V_1 + V_2$ in the model for thiosulfate treated dogs, were 498 and 204 ml/kg, respectively.

Pulmonary Excretion

Less than one percent of the dose of cyanide was eliminated via the lungs in both control and thiosulfate treated animals (Table 6.4). Most of the cyanide recovered from expired air was collected during the first minute after cyanide was administered.

Respiratory acidosis was noted in the blood of control animals but not in those treated with thiosulfate (Table 6.5). A peak pO_2 was observed in both groups 1 minute after cyanide was administered. This peak coincided with a peak in the respiration rate (Tables 6.4 and 6.6).

DISCUSSION

Thiocyanate distributed ultimately throughout a space that had an apparent volume of 47% of body weight. The compound dispersed rapidly after injection into an apparent volume of 40% of body weight, with complete

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TABLE 6.1

Pharmacokinetic Model Parameters $\pm$ S.D. for Thiocyanate
Alone and in Thiosulfate Treated Dogs

Model Parameter	Control	With Thiosulfate
V_1 (ml/kg)	405 ± 4.69	402 ± 5.70
K_{12} (h^{-1})	$0.150 \pm .0151$	$0.148 \pm .0185$
K_{21} (h^{-1})	$0.941 \pm .0539$	$0.844 \pm .0627$
K_{10} (h^{-1})	$0.0283 \pm .00168$	$0.0330 \pm .00316$

TABLE 6.2

Correlation Coefficients (r) and Sums of Squares (ss)
for Fits of Cyanide Model Pharmacokinetics

	r	ss
Control		
C_{CN} (Eq. 4)	0.983	$.118 \times 10^{-3}$
C_c (Eq. 5)	0.988	$.812 \times 10^{-4}$
With Thiosulfate		
C_{CN} (Eq. 7)	1.00	$.340 \times 10^{-3}$
C_c (Eq. 5)	0.979	$.113 \times 10^{-3}$
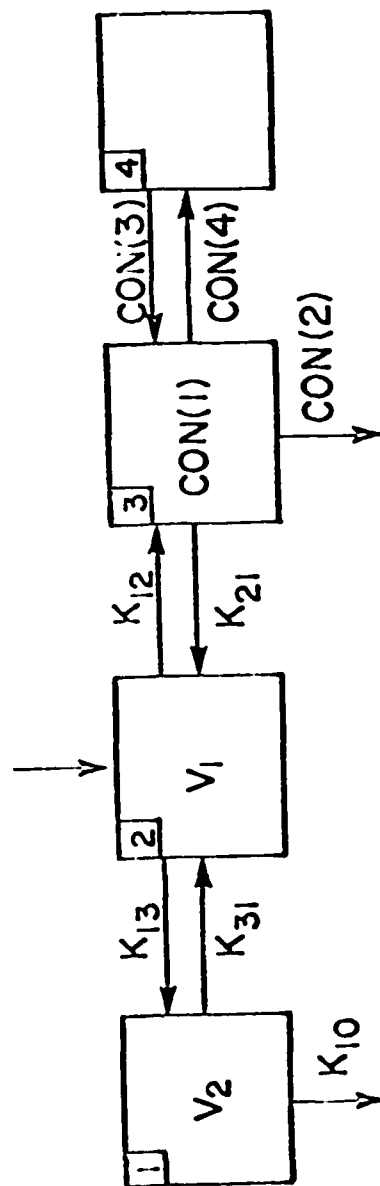
Fig. 6.9. Cyanide Pharmacokinetic Model of Distribution and Elimination (Thiosulfate Pretreated Dogs).

Con(1) refers to V_1 listed in Table 6.1.
Con(2) refers to K_{10} listed in Table 6.1.
Con(3) refers to K_{12} listed in Table 6.1.
Con(4) refers to K_{21} listed in Table 6.1.

Fig. 6.10. Model Predicted Blood Cyanide
Concentration and Observed Blood
Cyanide Concentration in Control
Dogs.
—— Model predicted using Eqs. 4-6
and parameters listed in Table 6.3.
● Experimentally determined

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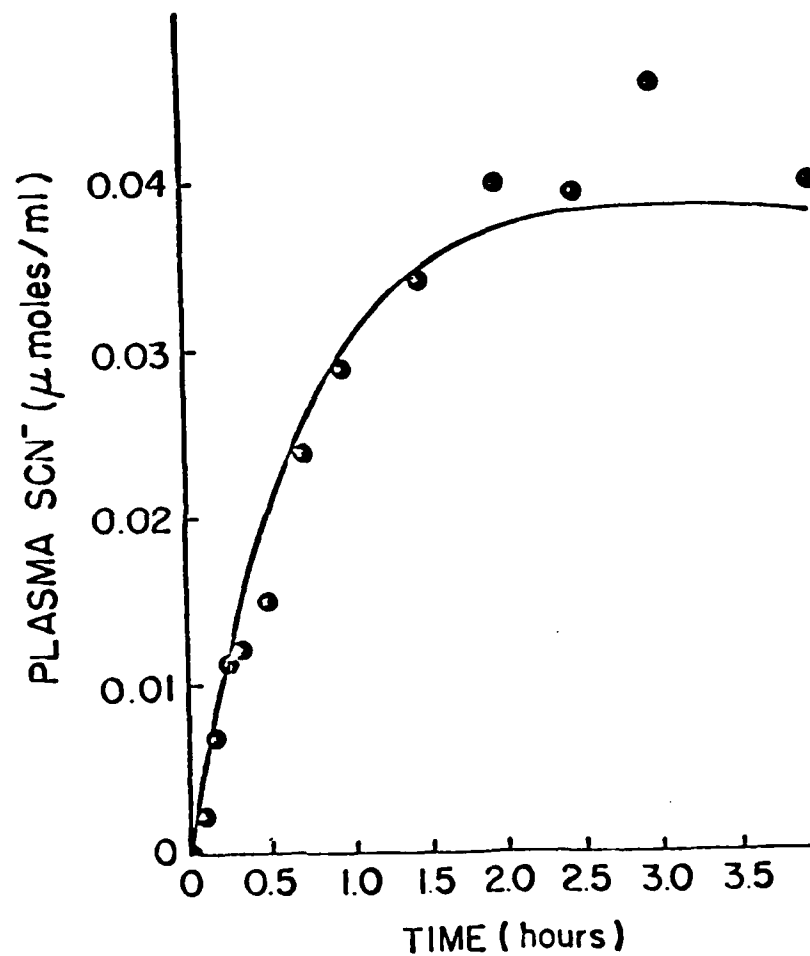
Fig. 6.11. Model Predicted Plasma Thiocyanate
Concentration in Control Dogs.
—— Model predicted using Eqs. 4-6
and parameters listed in Table 6.3.
● Experimentally determined

23
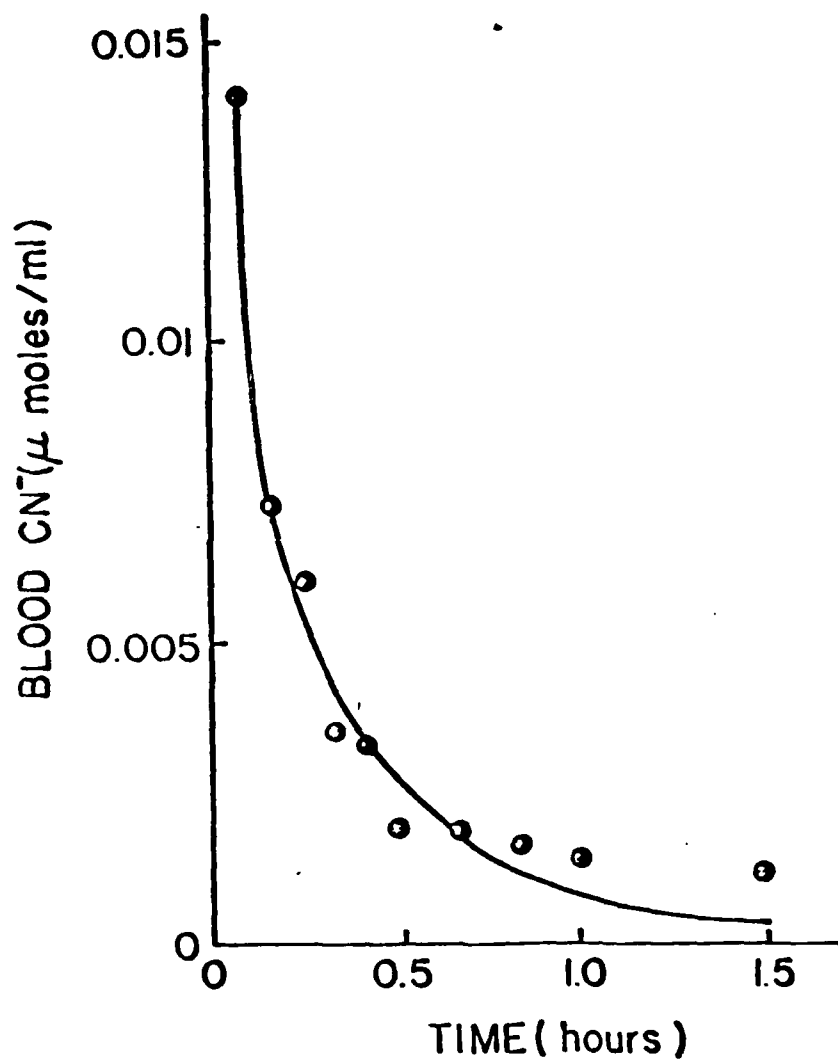
Fig. 6.12. Model Predicted Blood Cyanide
Concentration and Observed Blood
Cyanide Concentration in Thio-
sulfate Pretreated Dogs.
—— Model predicted using Eqs. 5-8
and parameters listed in Table 6.3.
● Experimentally determined

24

TABLE 6.3

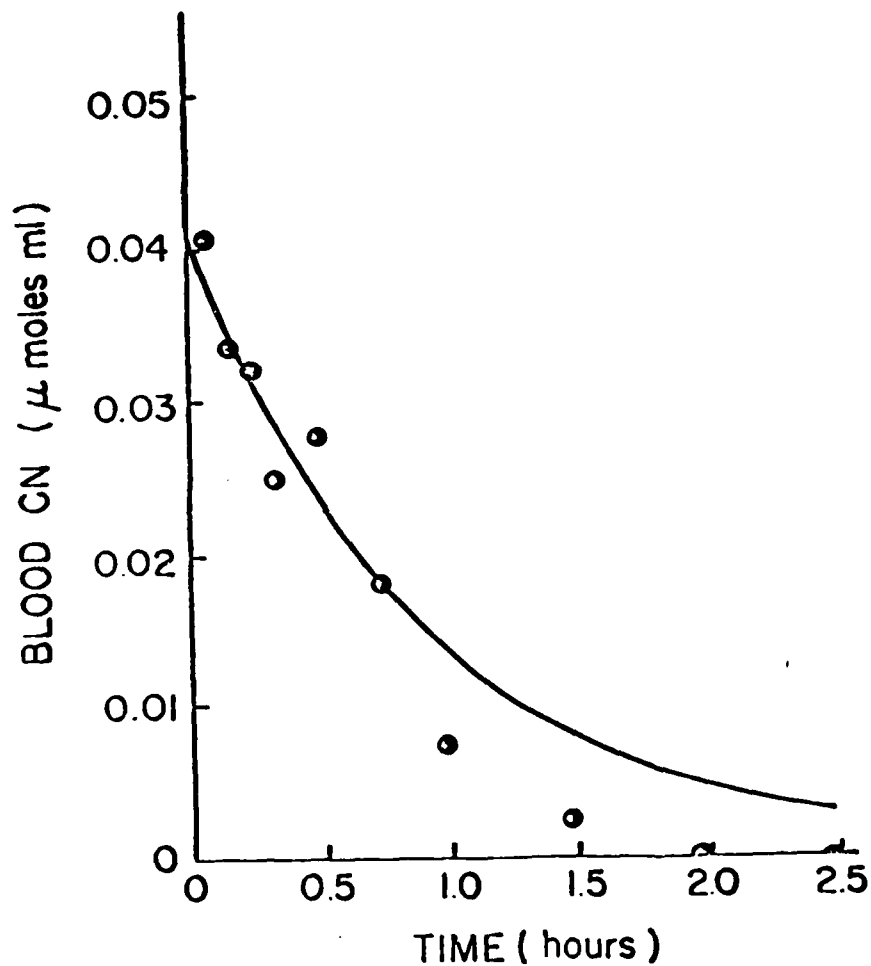### Cyanide Model Parameters

Parameter	Control	Thiosulfate Pretreated
K_{10} (hr^{-1})	$0.0568 \pm .0196$	$.253 \pm .0855$
K_{12} (hr^{-1})	$1.051 \pm .0894$	38.3 ± 4.19
K_{21} (hr^{-1})	$.0201 \pm .678 \times 10^{-2}$	$.182 \times 10^{-3} \pm .730 \pm 10^{-4}$
V_1 (ml/kg)	498 ± 22.4	39.2 ± 9.64
V_2 (ml)		165
K_{13} (hr^{-1})		15.6 ± 1.56
K_{31} (hr^{-1})		$3.70 \pm .536$
V_{total} (mg/kg)	498	204

TABLE 6.4

Amount of Cyanide Expired After I.V. Administration of Cyanide in Control and Thiosulfate Pretreated Dogs

Time (min)	μmoles CN^-	
	Control	S_2O_3
0	0.03	0.2
1	2.19	2.90
2	0.10	0.08
3	0.08	0.04
4	0.08	0.02
5	0.11	0.02
5 minute total	2.57	3.06
μmoles/kg	0.077	0.140
% dose	0.38	0.69

TABLE 6.5

Venous Blood Gases in Control and Thiosulfate Pretreated Dogs After Cyanide Administration

Time	pH		PCO_2		PO_2	
	Control	S_2O_3	Control	S_2O_3	Control	S_2O_3
0	7.389	7.397	34	39	48	42
1	7.368	7.442	32	32	87	68
2	7.331	7.370	36	36	54	51
3	7.308	7.342	39	47	47	48
4	7.288	7.332	39	40	42	45
5	7.291	7.350	38	38	39	38

TABLE 6.6

Respirations After Cyanide Administration in Control
and Thiosulfate Pretreated Dogs

| Time (min) | Minute Respiratory Volume (L) | | Breaths/Min | |
	Control	Thiosulfate	Control	Thiosulfate
0	2.90	2.72	10	12
1	6.55	7.0	15	22
2	4.65	1.65	14	10
3	5.44	2.80	12	11
4	5.95	4.05	12	14
5	7.20	4.50	14	14

distribution requiring about 3 hours. These values for the apparent volume of distribution of thiocyanate are slightly higher than previously published values in dog (Gaudino et al., 1948; Elkinton and Taffel, 1942). Since the extracellular fluid volume in the dog is generally considered to be about 25% of body weight (Altman, 1961), thiocyanate does not appear to be a good compound for measuring this parameter. The total body clearance of thiocyanate averaged about 12 ml/hr/kg, much less than the glomerular filtration rate, which is generally accepted to be 84 ml/min/m^2 in the dog (Altman, 1961). This low clearance probably results from plasma protein binding of thiocyanate.

The model that was used for thiocyanate appeared to fit the measured thiocyanate plasma concentrations reasonably well (Figs. 6.4 and 6.5). The data scattered randomly about the model predicted lines and correlation coefficients were greater than 0.99. The presence of thiosulfate did not significantly alter any of the pharmacokinetic model parameters for thiocyanate and it therefore appeared reasonable to assume that thiosulfate did not alter the distribution or elimination of thiocyanate formed from cyanide.

In the absence of thiosulfate, cyanide and thiocyanate concentrations in blood and plasma after cyanide administration indicated that cyanide disappeared relatively rapidly with simultaneous appearance of thiocyanate. The apparent volume of distribution of cyanide was about 50% of body weight and cyanide appeared to distribute rapidly throughout this volume. The first sample of blood was removed 5 minutes after i.v. injection of cyanide and distribution appeared to be complete by that time since the blood concentration of cyanide declined monoexponentially with time. The model that was used for cyanide and thiocyanate (Fig. 6.8), adequately mimiced the observed behavior of these compounds in the blood and plasma (Figs. 6.10 and 6.11). While cyanide and thiocyanate were modeled as an equilibrium, a simulation

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that used the model and parameters in Table 6.3 showed that for practical purposes the equilibrium between cyanide and thiocyanate was not achieved. In the simulation, the concentrations of the compounds reached a constant ratio only after 3 hours. By that time, 90% of the dose of cyanide had been converted to thiocyanate. Thiocyanate therefore does not appear to act as a reservoir for cyanide and does not appear to limit the elimination of cyanide. This is consistent with the thermodynamic data of rhodanese (Sorbo, 1953) and the role of thiocyanate oxidase (Goldstein and Rieders, 1951). The simulation also showed that most of the cyanide was converted to thiocyanate (Fig. 6.14). Less than 8% of the dose of cyanide was predicted to disappear by the non-thiocyanate route, K_{10} in Fig. 6.8. The clearance for the conversion of cyanide to thiocyanate, $K_{12}V_1$, was 523 ml/hr/kg. Conversion of cyanide to thiocyanate is believed to occur primarily in the liver. Hepatic blood flow in the dog is approximately 1500 ml/hr/kg (Green, 1950) and it appears, therefore, that the hepatic extraction ratio of cyanide is about 35%. This fraction of orally or intraperitoneally administered cyanide could be removed by a "first-pass" mechanism, leading to an apparently higher LD_{50} for these routes compared to other routes.

Thiosulfate dramatically altered the blood level-time behavior of cyanide and thiocyanate, relative to the control. Examination of the blood concentration-time data for cyanide, Fig. 6.6, led to the suggestion that thiosulfate acted only to increase the apparent volume of distribution of cyanide, since the blood concentration-time values in the thiosulfate treated animals were lower but parallel to the values observed in the control animals. However, altered distribution was not consistent with the more rapid appearance of thiocyanate in thiosulfate treated animals compared to the controls, Fig. 6.7. The difference between the initial blood concentrations

of cyanide in thiosulfate treated and control animals could be accounted for by the relatively large differences in the amounts of thiocyanate that were present. Analysis of the cyanide and thiocyanate data led to the development of a model (Fig. 6.9) that was consistent with the data (Figs. 6.12 and 6.13). With this model, cyanide is converted very rapidly to thiocyanate in a relatively small volume, probably the blood. Simulations with the model showed that more than 50% of a dose of cyanide is converted to thiocyanate within 3 minutes of cyanide administration (Fig. 6.14). Along with this rapid conversion to thiocyanate, some cyanide distributes extravascularly, leading to a relatively slow rate of elimination of cyanide after this distributive phase ends. The distributive phase was predicted to end before the first blood sample was taken at 5 minutes, explaining why cyanide blood concentration-time data in control and thiosulfate treated animals appeared to be parallel during this post-distributive phase. While the clearance of cyanide to thiocyanate ($K_{12}V_1$, Fig. 6.9) is increased only by a factor of 3 (from 523 to 1500 ml/hr/kg) in thiosulfate treated animals, the volume of the compartment in which clearance occurs is much smaller in thiosulfate treated animals compared to controls. The initial rate of thiocyanate formation is therefore increased by a factor of 36.4 in thiosulfate treated animals compared to controls (Table 6.3). As in the control model, the model shown in Fig. 6.9 predicts that the reversion of thiocyanate to cyanide is not a significant determinant of the blood concentration of cyanide, and the elimination of cyanide from compartment 1 accounts for less than 3% of the dose. Because the apparent volume of distribution of the compartment in which cyanide is converted to thiocyanate is approximately equal to the blood volume, and because the initial rate of conversion of cyanide to thiocyanate is very rapid, it appears likely that cyanide is converted to thiocyanate primarily in the blood. If the blood is
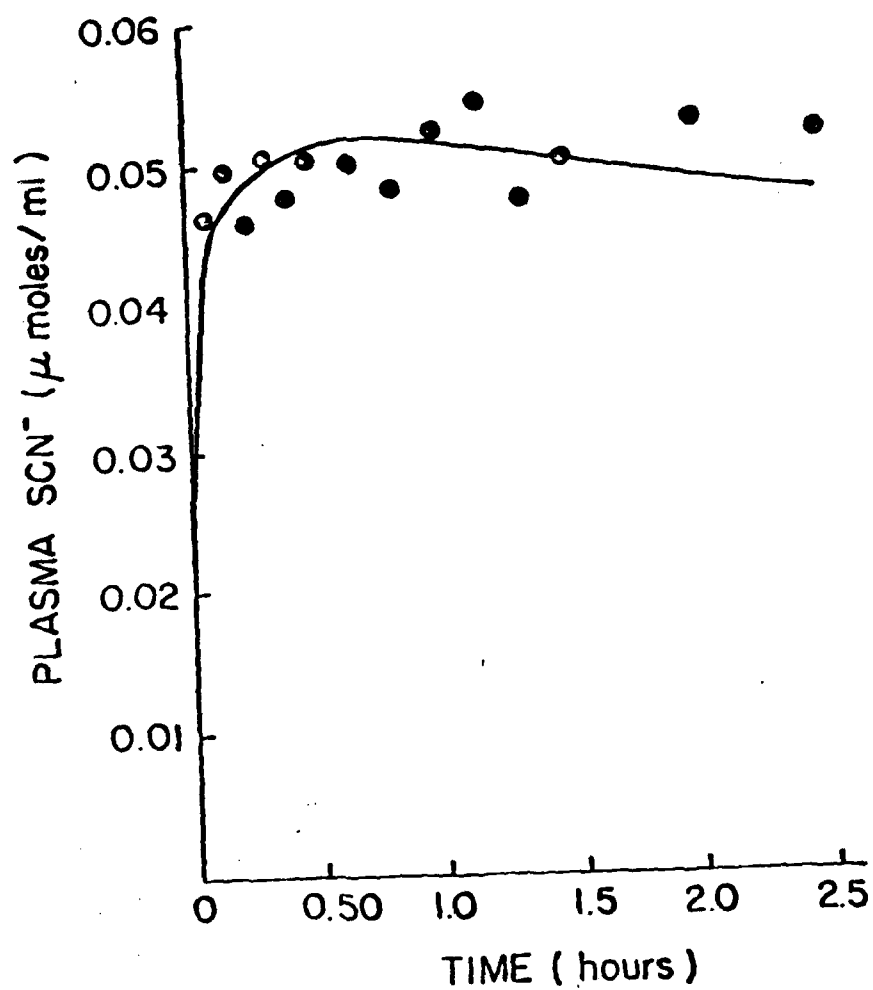
31

Fig. 6.13. Model Predicted Plasma Thiocyanate
Concentration and Observed Plasma
Thiocyanate in Thiosulfate F -
treated Dogs.
—— Model predicted using Eqs. 5-8
and parameters listed in Table 6.3.
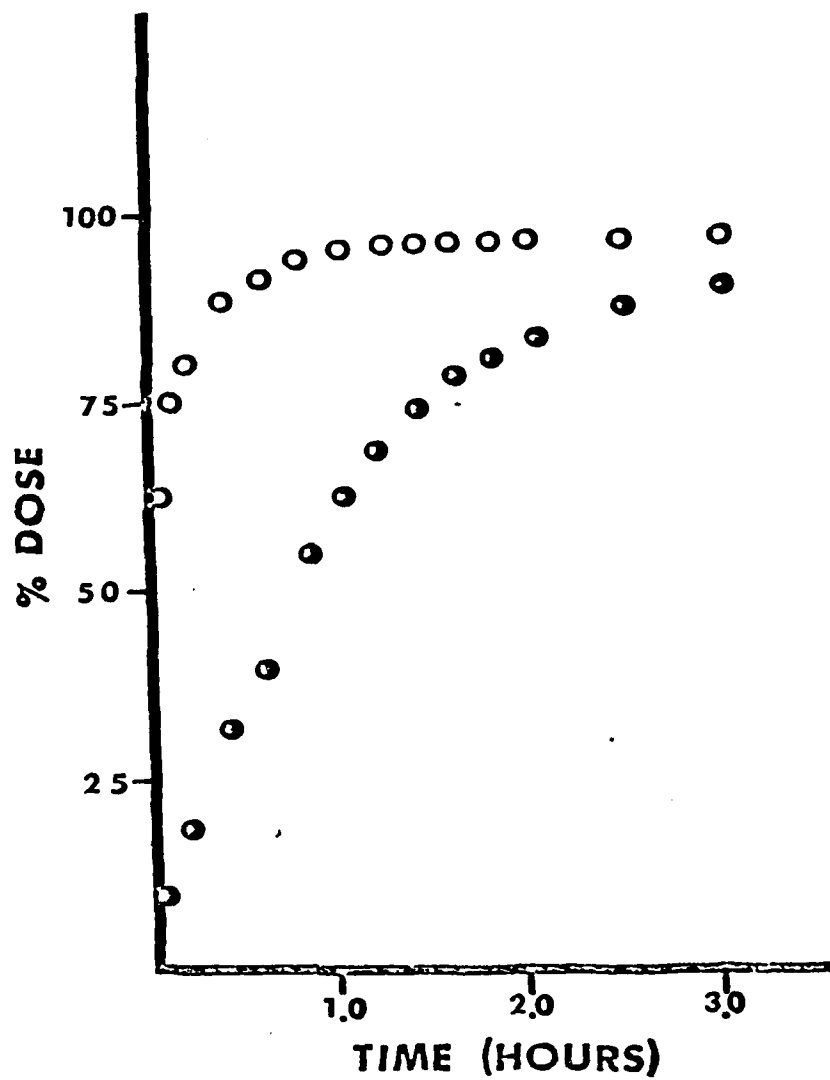● Experimentally determined

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Fig. 6.14. Model Predicted % Cyanide Dose
Converted to Thiocyanate.
•, Control dogs
o, Thiosulfate pretreated dogs

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the site for this conversion, thiosulfate probably does not exert its initial major antidotal effect by serving as a source of sulfur for extraerythrocytic rhodanese. However, it should be pointed out that rhodanese has a turnover number of over 20,000 (Sorbo, 1953), and is distributed in high concentrations in many highly perfused organs (Himwich and Saunders, 1948) in close proximity to blood; therefore, the contribution of extraerythrocytic rhodanese may be more important than kinetically apparent.

Pulmonary excretion of cyanide after its i.v. administration was considered to be a potentially important route since cyanide is volatile and most of an i.v. dose would pass through the lungs before the compound distributed into other tissues. The results from this study indicated that pulmonary excretion was not a significant route of elimination for cyanide, and the antidotal effect of thiosulfate did not result from its effect on the rate of respiration. These pulmonary excretion data are consistent with studies reported in mice (Burrows et al., 1976).

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PART II

EFFECT OF THIOSULFATE ON THE POTENTIOMETRIC DETERMINATION

OF CYANIDE AND ITS ELIMINATION

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OF CYANIDE AND ITS ELIMINATION

INTRODUCTION

Cyanide intoxication is of widespread occurrence, as it can be derived from a variety of medicinal, dietary, environmental and industrial sources (Heaton et al., 1958; Herrington et al., 1971; Vesey et al., 1976; Michenfelder and Tinker, 1977; Contessa et al., 1977). The classic method employed to treat cyanide poisoning is to use a combination of sodium nitrite and sodium thiosulfate (Chen and Rose, 1952). Although there have been numerous reports on determining cyanide in biological fluids, many of these studies have over-looked the possible effects of various cyanide antagonists, e.g., sodium thiosulfate, on interfering with the analytical procedures to measure cyanide. This is of considerable importance, as the accurate evaluation of the efficacy of these antidotes and the improvement of the subject poisoned with cyanide is dependent on these types of investigations.

There are a variety of different methods to measure cyanide in bio-logical fluids and, as in every method, there are limitations with regard to their sample size, sensitivity and chemicals which may interfere with the method being employed. In adapting most analytical methods to determine cyanide in biological fluids, a preliminary gaseous diffusion or microdif-fusion procedure is usually employed by acidification of the sample so that the cyanide not only can be concentrated, but non-volatile chemicals which can

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interfere with the determination of cyanide can be separated from cyanide. In spite of these diffusion procedures, one of the cyanide antagonists, sodium thiosulfate has presented problem, as it produces interference with some of the colorimetric and fluorometric methods usually employed to measure cyanide (Morgan and Way, 1979; Morgan et al., 1979). This interference has been attributed to the formation of polythionic acids from sodium thiosulfate upon acidification of the sample. The polythionic acid then decomposes to liberate predominantly sulfur dioxide and is trapped as sulfite. This latter anion inhibits the colorimeteric (Epstein, 1947) and fluorometric (Morgan and Way, 1979; Morgan et al., 1979) methods.

Recently the potentiometric determination of cyanide has become the state of the art method, as it is a convenient and highly sensitive method (Blaedel et al., 1971). Moreover, sulfite ion does not interfere with the silver/sulfide ion selective eletrode, and thereby may provide a basis to determine cyanide in the presence of sodium thiosulfate. Unfortunately, as indicated in these studies, the potentiometric analysis of cyanide in the presence of thiosulfate erroneously elevates the value for cyanide. The chemical basis for this interference is based upn the degradation of poly-thionic acids to a complex series of products which are formed, including a small amount of sulfides (Roy and Trudinger, 1978). Although the percentage and amount of sulfide formed from thiosulfate is relatively small, the silver/sulfide electrodes are highly sensitive to sulfide and therefore the inter-ference is considerable.

These studies indicate that sulfide derived from sodium thiosulfate is the interfering anion in the potentiometric analysis of cyanide and this interference is eliminated by the oxidation of the sulfide with hydrogen peroxide.

MATERIALS AND METHODS

Materials

The chemicals used in this study were analyzed reagent grade or of the highest purity available. Sodium thiosulfate, hydrogen peroxide, sodium mono- and dibasic phosphates, sodium hydroxide, sodium cyanide and sodium acetate were obtained from J. T. Baker Chemicals (Phillipsburg, NJ) and ethylenediamine was obtained from Eastman Organic Chemicals (Rochester, NY), and redistilled before use. Sodium dodecyl sulfate (SDS) was purchased from Sigma Chemical Co. (St. Louis, MO) and silver potassium cyanide was a product of K and K Laboratories, Inc. (Plainview, NY). Sodium heparin was a product of A. H. Robins, Co. (Richmond, VA).

Methods

Cyanide determinations were performed after microdiffusion analysis using a silver/sulfide ion specific electrode and a Model 701-A digital pH meter (Orion Research, Inc., Boston, MA).

A 2.0 ml aliquot of 0.1 N NaOH was placed in the center well of a Conway microdiffusion cell (A. H. Thomas Co., Philadelphia, PA) and 0.5 ml of 10^{-4} M SDS and 1.0 ml of sample was added to the outer well. Immediately after the addition of 1.0 ml of 1.0 M acetate buffer, pH 5.2, to the outer well, the cells were sealed with ground glass covers coated with lubriseal (A. H. Thomas Co.). Cells were rotated initially and at 30 minute-intervals for three hours to insure proper mixing and diffusion. After diffusion was completed, 0.5 ml of 0.1 N NaOH was removed from the center well of the diffusion cell and added to a microsampling dish (Orion Research, Inc.) containing 10 µl of a silver potassium cyanide indicator-buffer solution (17.69 g Na_2HPO_4, 5.5 ml 10 M NaOH, 10 mg silver potassium cyanide indicator, and 3.3 ml ethylenediamine/100 ml). The potential was determined while the sample was

gently agitated. Cyanide concentrations were extrapolated from a standard curve which was determined daily.

In order to eliminate thiosulfate interference in the cyanide assay, the center well of the diffusion cell was treated just prior to electrode analysis. The cell was opened and 0.2 ml of 1.0 M hydrogen peroxide was added to the center well and mixed gently. Two minutes later, 0.2 ml of 1 M sodium sulfite was added to the center well, mixed and 0.5 ml of the treated sodium hydroxide was analyzed for cyanide with the ion specific electrode. For comparison, untreated samples received equal volumes of 0.1 N NaOH instead of hydrogen peroxide and sodium sulfite.

In the whole blood studies, fresh heparinized dog blood was used and equilibrated at 37°C. Sodium thiosulfate or saline control was mixed with the blood and equilibrated for fifteen minutes at 37°C and then sodium cyanide was mixed with the samples. After temperature equilibration the blood was analyzed for cyanide concentration.

Sodium sulfite was examined for possible interference, since thio-sulfate can be degraded to sulfite. Increasing amounts of sulfite were added to a constant cyanide concentration and then analyzed for cyanide. A sulfide standard curve also was determined, and subsequently treated with hydrogen peroxide and sodium sulfite to show removal of the interfering compound.

RESULTS

The effect of sodium thiosulfate on increasing concentrations of sodium cyanide is shown in Fig. 1. The presence of a constant amount of sodium thio-sulfate increases the apparent cyanide concentration by a constant amount as is evidenced by a fixed increase in millivolt potential of the standard curve for cyanide. The only difference between the curves is the presence of sodium
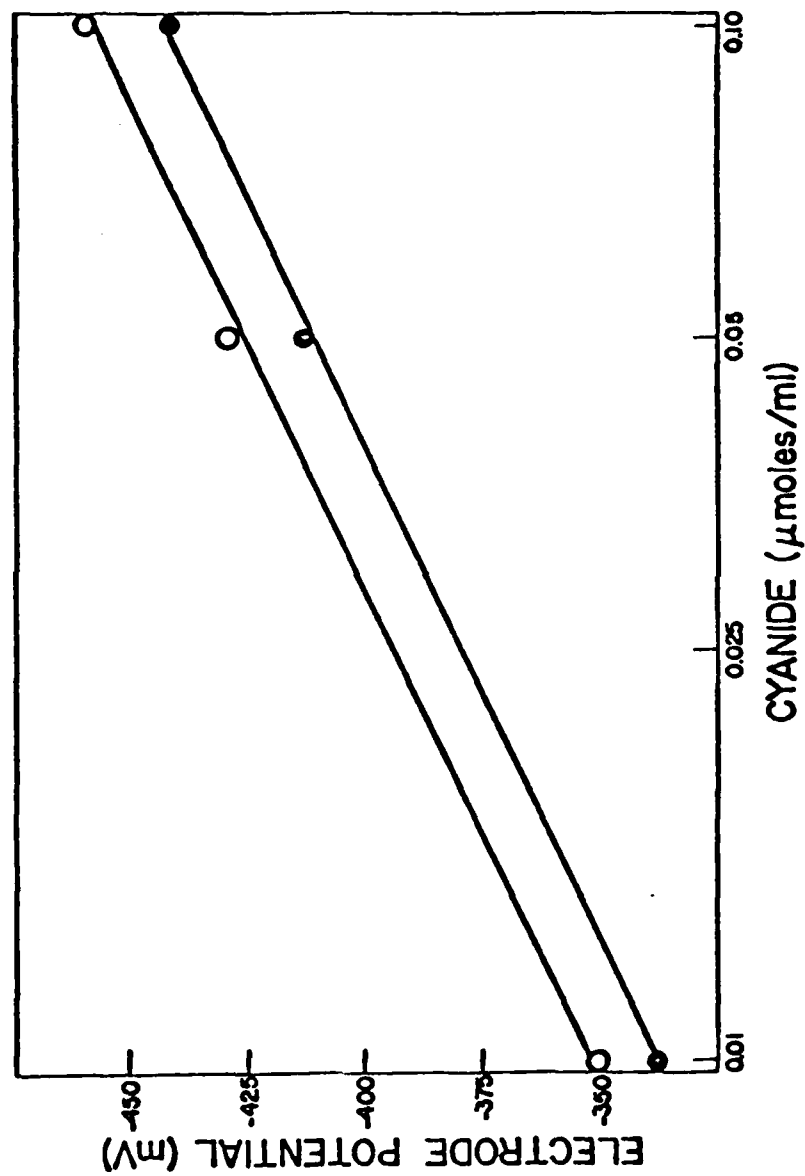
Fig. 3.1. Sodium Cyanide Millivolt Potential with and without Sodium Thiosulfate.

o—o, with thiosulfate (32 μmole/ml)

●—●, without thiosulfate

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thiosulfate in the standard curve eliciting the higher electrode potential even though both curves are parallel throughout. Also, it should be pointed out that when the higher curve containing the sodium thiosulfate is treated with hydrogen peroxide and subsequently sodium sulfite, the resulting curve is identical to the lower curve which contained no sodium thiosulfate.

The effect of increasing concentrations of sodium thiosulfate on cyanide determination in saline and whole blood is shown in Fig. 2. These studies were conducted in isotonic saline and whole blood, as even without sodium thiosulfate there were small increases in electrode potential with increasing amounts of blood. The apparent cyanide appears to be enhanced with an increase in sodium thiosulfate, and the increased interference was directly proportionate to the amount of sodium thiosulfate. It is of interest to note that the increase in electrode potential as indicated by "apparent cyanide" is proportionately higher when whole blood is employed, as is manifested by the steeper slope in whole blood. Since the two curves are not parallel, increases in sodium thiosulfate in whole blood produces a disproportionately higher enhancement of the interference when compared to isotonic saline. The difference in slopes is not due to an interaction of cyanide with sodium thiosulfate or blood, as separate studies done with cyanide gave identical curves. Furthermore, increasing blood concentration produced a proportionate increase in the positive interference.

Figure 2 shows the effects of sodium thiosulfate on cyanide concentration in aqueous systems and in whole blood. A constant amount of cyanide appears to increase as the amount of thiosulfate increases. Increased cyanide levels are seen with concentrations of thiosulfate as low as 4 μmoles/ml in whole blood sampels, and 8 μmoles/ml in aqueous samples. The interference in aqueous samples is thought to result from the liberation of sulfide from
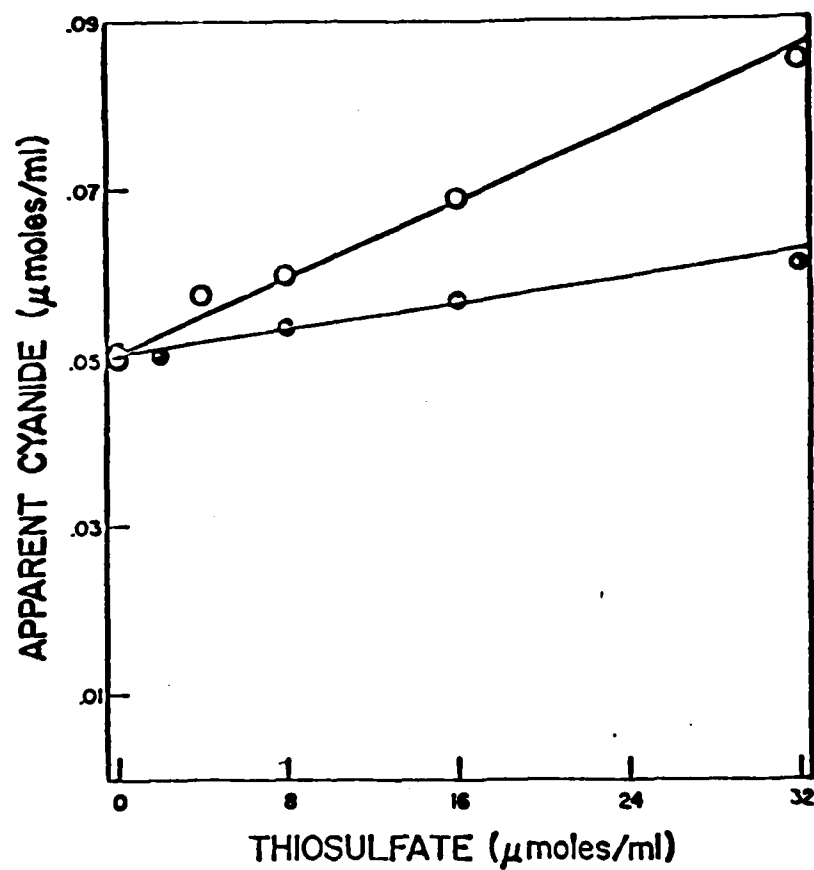
Fig. 3.2. Sodium Cyanide (0.05 μmole/ml)
Determination in Isotonic Saline
with Sodium Thiosulfate and in
Whole Blood with Sodium Thiosulfate.
o——o, whole blood
•——•, isotonic saline

thiosulfate under the acid conditions of the outer well of the microdiffusion cell. The interference seen in whole blood samples is greater than in aqueous samples. The difference is attributed to the formation of sulfide from both the acid conditions of the microdiffusion and from thiol reduction of thiosulfate to sulfide during blood incubation with thiosulfate.

Treatment of aqueous and whole blood samples containing sodium cyanide with hydrogen peroxide and subsequently sodium sulfite resulted in the elimination of the positive interference produced by increasing amounts of sodium thiosulfate in aqueous (Fig. 3) and in whole blood (Fig. 4) samples. Aqueous samples containing up to 64 μmoles/ml of sodium thiosulfate do not interfere in the cyanide analysis after samples are treated with hydrogen peroxide and sodium sulfite. More important, in whole blood containing up to 32 μmoles/ml of sodium thiosulfate (Fig. 4), the interference was resolved by these procedures, as is evidenced by the fact that the apparent cyanide concentration remained constant in the presence of increasing concentrations of sodium thiosulfate.

It is important that the influence of sulfite on the potentiometric analysis of cyanide be assessed, as this anion represents the predominant volatile product formed as sulfur dioxide by the decomposition of polythionic acids and is the method employed to remove the excess hydrogen peroxide. Furthermore, sulfite has been reported to interfere with the colorimetric (Morgan and Way, 1979) and fluorometric (Morgan et al., 1979) analysis of cyanide. The sulfite ion even in even high concentrations exhibited no effect on the potentiometric determination of cyanide with the silver/sulfide electrodes, as the cyanide concentration remained constant with increasing concentration of sodium sulfite (Fig. 5).

Fig. 3.3 Sodium Cyanide (0.05 μmole/ml) in
Isotonic Saline Before and After
Treatment with H_2O_2 (0.2 mmole) and
Na_2SO_3 (0.2 mmole).
●——●, before treatment
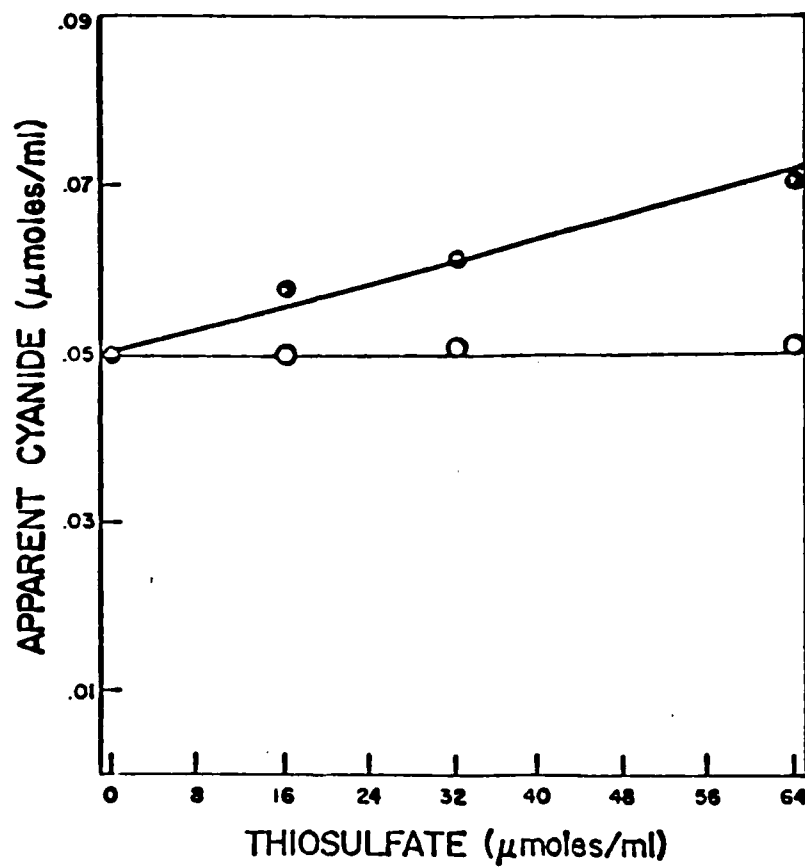o——o, after treatment

45
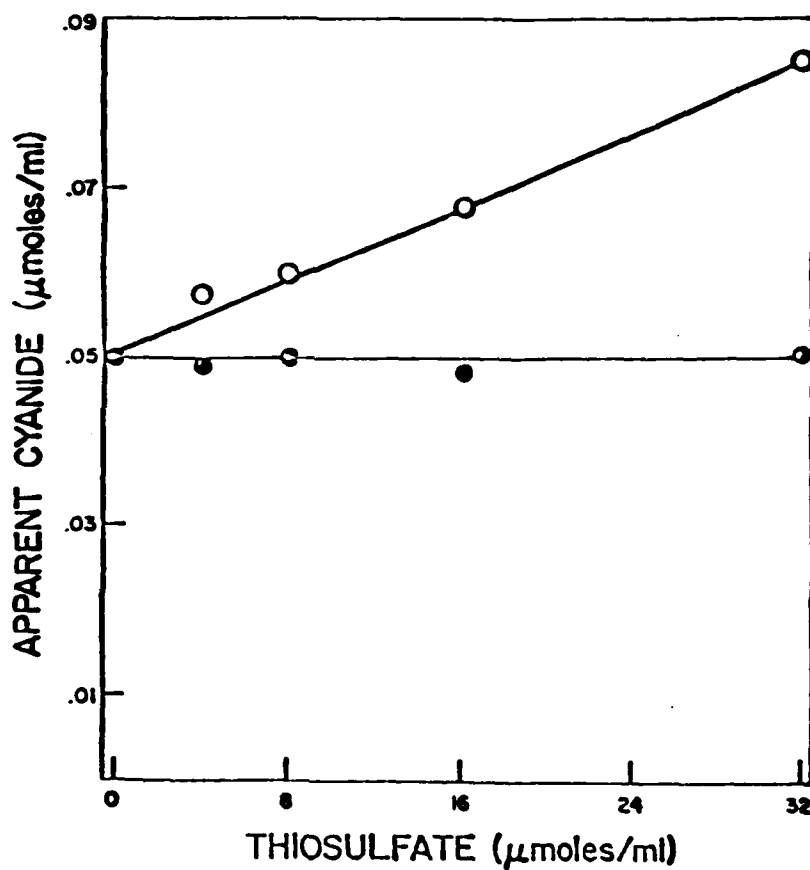
Fig. 3.4. Whole Blood with Sodium Cyanide (0.05
µmole/ml) and Sodium Thiosulfate Before
and After Treatment with H_2O_2 (0.2
mmole) and Na_2SO_3 (0.2 mmole).
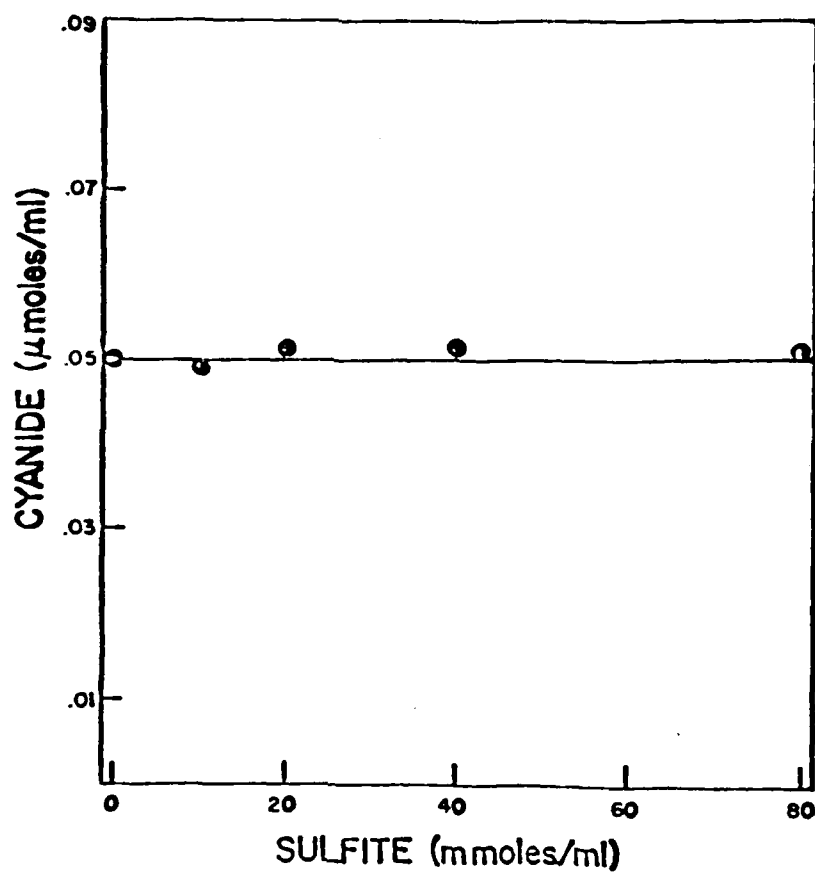o——o, before treatment
●——●, after treatment

Fig. 3.5. Sodium Cyanide (0.05 μmole/ml) in the Presence of Sodium Sulfite.

Since it was proposed that sulfide was the chemical species formed from sodium thiosulfate which interfered with the potentiometric analysis of cyanide, the effect of sodium sulfide on the silver/sulfide electrodes was conducted. As would be anticipated, the silver/sulfide electrode is very sensitive to sulfide ion, and very small concentrations of sulfide ion elicited large changes in the electrode potential (Fig. 6). More important, sulfide ion in the sample can be removed by oxidation with hydrogen peroxide. Also, the limit of sulfide ion which can be completely oxidized under the conditions of these investigations is 0.003 μmoles of sulfide, but on a practical basis it can oxidize 0.02 μmoles/ml or more of sulfide, as the potentiometric scale is logarithmic; therefore, the amount of sulfide shown after hydrogen peroxide is relatively trivial in these types of studies.

DISCUSSION

Although the potentiometric determination of cyanide in biologic fluids is a convenient, rapid and sensitive method to measure cyanide, there are limitations in its use, particularly in the presence of the cyanide antagonist, sodium thiosulfate. Subsequent to the early reports on the use of silver/sulfide electrodes for the measurement of cyanide (Frant et al., 1972), it quickly became the method of choice in many laboratories for measuring cyanide in biologic fluids. There are some reports on the limitations on the potentiometric analysis of cyanide in biologic fluids; however, those studies used the ion specific electrodes directly in biologic fluids without prior diffusion procedures to remove some of the interfering substances (Kistner et al., 1979).

In the colorimetric and fluorometric methods the predominant inter- ference was found to be due to sulfite formation; however, this could not be
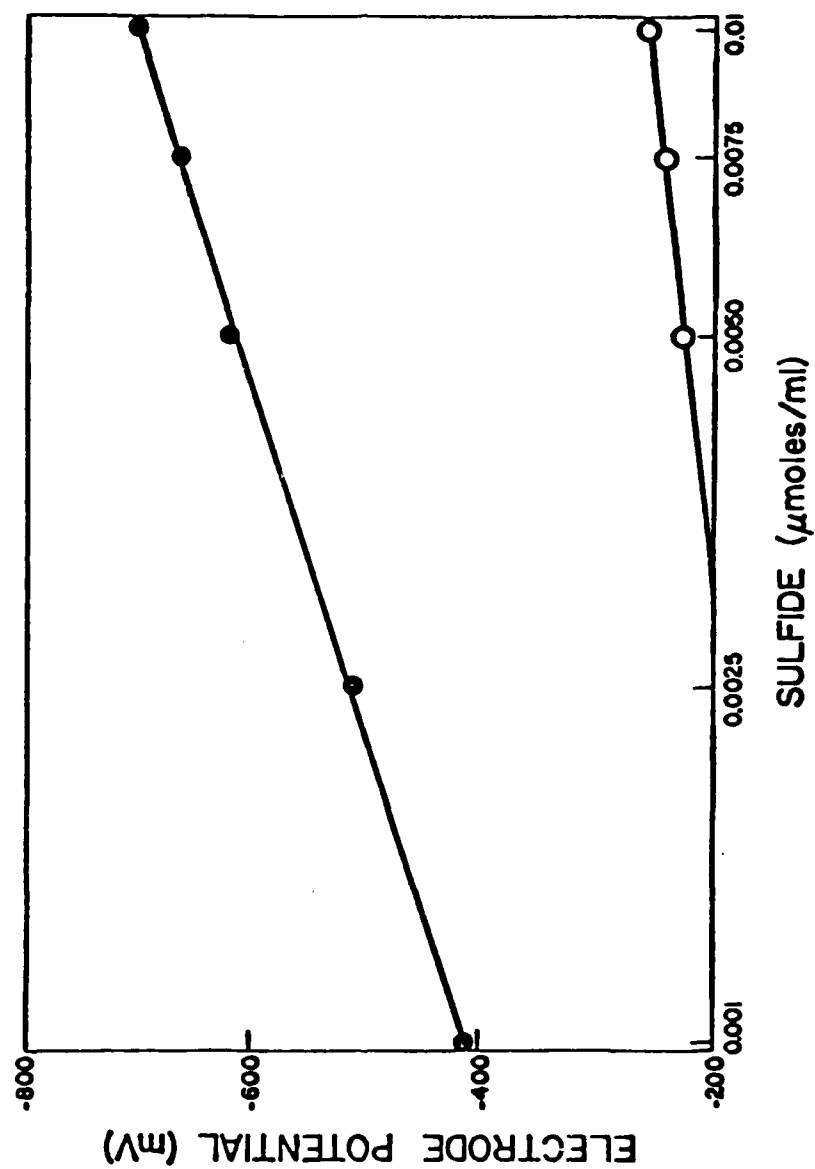
Fig. 3.6. Sodium Sulfide Determination Before and After Treatment with H_2O_2 ().2 mmole) and Na_2SO_3 (0.2 mmole).

●———●, before treatment
○———○, after treatment

the basis with the potentiometric analysis, as sulfite is not supposed to interfere with the potentiometric cyanide analysis. Therefore, an alternative chemical basis must be provided for the positive interference produced by sodium thiosulfate in the potentiometric determination of cyanide in biologic fluids. One of the chemical bases proposed is that upon the acidification of sodium thiosulfate, a series of fairly complicated reactions occur, and the decomposition products formed are dependent on various conditions, particularly the acidity of the media (Nickless, 1968; Karchmar, 1970). The thiosulfate anion is converted to polythionic acids with an empirical formula of $H_2S_nO_6$. These free acids formed are relatively unstable at room temperature, and degrades by a series of fairly complex reactions. For example, tetrathionate, which is formed initially, is rapidly degraded to trithionate and penta- thionate. Because of the electrophilicity of the divalent sulfur atom, they will react with various nucleophiles, forming other thionic acids. Also, a nucleophilic attack on a sulfur chain can destroy the thionic acid, liberating sulfur dioxide and sulfide ion. In most acidification media employed in the microdiffusion of hydrogen cyanide by Conway microdiffusion cells, the volatile gases are trapped in the center well which contains sodium hydroxide. Sulfite is one of the major anions which is formed; however, a small amount of sulfide is formed. The determination of cyanide in the presence of sodium thiosulfate is relatively complex, as the decomposition products formed interfere with different analytical methods to measure cyanide by different mechanisms. For example, it is the liberation of sulfur dioxide which is ultimately trapped as sulfite, which interferes with the colorimetric method (Morgan and Way, 1979) and the fluorometric (Morgan et al., 1980) method. It is probably the forma- tion of small amounts of sulfide which interferes with the potentiometric determination of cyanide with the silver/sulfide electrodes. Although hydrogen

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sulfide is a common contaminant when cyanide is being measured, e.g., on gas analysis from coke ovens (Manka, 1975), these methods cannot be employed when micro amounts of sulfide and cyanide are being measured in biologic fluids. The initial attempt to remove sulfide in the microdiffusion procedure was to add lead acetate; however, under our conditions the lead acetate procedure to remove sulfide produced low values. In our hands this was not a consistently reliable method to measure cyanide in the presence of hydrogen sulfide. However, it should be pointed out that lead acetate has been employed by other laboratories (Egekeze and Oehme, 1979; McAnalley et al., 1979).

The initial results when measuring cyanide in standard solutions with and without sodium thiosulfate (Figure 1) present a rather simplistic problem with regard to the positive interference produced by sodium thiosulfate. There is a finite amount of sulfide formed which will produce a quantitatively predictable interference with cyanide analysis. However, comparing these studies both in aqueous solution and blood presents a rather different picture. With increasing concentrations of sodium thiosulfate it is readily apparent that the slope for whole blood is much steeper than that with aqueous solution. This would suggest that there is a varying amount of sulfide being liberated from a constant amount of sodium thiosulfate, depending on whether aqueous solutions or blood was employed. These figures have not been shown in the results; however, it was important to establish that the artifact produced by sodium thiosulfate was not related to interaction with cyanide. Additional studies conducted without the presence of cyanide obtained results similar to that with cyanide, as the response was linear. In other studies where the sodium thiosulfate was held constant and the amount of blood was varied, the increase in blood concentrations produced a rather steep convex curve, whereas with isotonic saline, a much lower linear curve was observed. These ⁴.

would suggest that blood enhances the conversion of sulfide from thiosulfate by an enzymatic reaction. This is not unreasonable, as thiosulfate in mammalian systems is rapidly oxidized to sulfate from the inner sulfur atom of thiosulfate, whereas the outer sulfur atom enters into a pathway where the mechanism of metabolism is not clear.

The thiosulfate sulfur transferase must be present in blood to produce a reductive cleavage of thiosulfate in the presence of other sulfur compounds; however, there are reports which suggest that the initial step of thisulfate oxidation in mammals is thiosulfate reductase, which catalyzes the interaction of thiosulfate with glutathione to form sulfite, hydrogen sulfide or dihydro- lipoate as the thiol RSH (Jocelyn,1972; ~~Greenberg, 1975~~). The reaction would be as follows:

$$S_2O_3^{2-} + 2\ RSH \rightarrow SO_3^{2-} + H_2S + RSSR$$
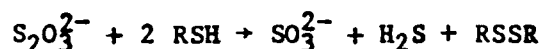
However, regardless of the mechanisms involved and the amount of interference produced, the use of hydrogen peroxide to oxidize these sulfides is quite effective, as the interference produced in isotonic saline and in whole blood can be completely removed.

Since the formation of sulfite from sodium thiosulfate has caused con- siderable interference of many of the analytical methods to measure cyanide, it was felt essential to establish firmly that sulfite formed by the degrada- tion of thiosulfate would not interfere with this potentiometic method. Also, this would justify the use of sulfite to remove the excess hydrogen peroxide. The inability of sulfite to interfere with the potentiometric analysis of cyanide at a wide concentration range was indicated in these studies. The sulfur chemistry reported by other laboratories would infer that the positive interference produced in this analytical method by sodium thiosulfate was

probably due to the formation of sulfide. Therefore, it was necessary not only to demonstrate the extreme sensitivity of sulfide ion to the silver/sulfide electrode, but to emphasize that only a small contamination was necessary to produce a substantial positive interference of cyanide. Also, these studies clearly indicate that hydrogen peroxide was effective, not only in removing the sulfide contamination, but also that the inactivation of hydrogen peroxide with sulfite occurs without apparent complications.

In summary, this investigation indicates that sodium thiosulfate can produce a positive interference with cyanide determination to varying degrees in aqueous solutions and whole blood. This can produce rather erroneous interpretations of cyanide concentrations leading to wrong conclusions with regard to the efficacy of various antidotes employed in the treatment of cyanide poisoning. The amount of sulfide formed from sodium thiosulfate will vary according to not only the amount of thiosulfate, but also the amount of blood and this interference can be eliminated by the use of hydrogen peroxide.

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Part III

ANTAGONISM OF CYANIDE INTOXICATION

(Published in <u>Trends in Pharmacological Science</u>)

INTRODUCTION

There are a variety of antagonists which are quite efficacious and
this has lead to some differences of opion with regard to the treatment
of cyanide poisoning. The use of sodium nitrite and sodium thiosulfate to
treat cyanide poisoning was developed almost fifty years ago (Chen et al.,
1933) and this antidotal combination, because it was developed on a rational
pharmacological basis, is still employed in antagonizing cyanide poisoning.
This antidotal combination has been enhanced by the use of oxygen (Sheehy
and Way, 1968) as it potentiates the effectiveness of the sodium nitrite-
sodium thiosulfate antidotal combination.

The mechanism of cyanide intoxication is attributed to a rather
complex inhibition of cytochrome oxidase, the terminal oxidative respira-
tory enzyme in the mitochondria encatalysis the tissue utilization of
oxygen. This produces a histotoxic anoxia and aerobic metabolism is
inhibited. The underlying toxicologic mechanism is that the tissue have
sufficient oxygen but are unable to utilize this oxygen in tissue metabo-
lism. Most of the toxic mechanism attributed to cyanide has focused on
cytochrome oxidase because of the unusual sensitivity of this enzyme to
cyanide, but also because this enzyme is localized rather critically in the
biochemical metabolic chain. Although cyanide is well recognized for its
rapid lethal action, it is not usually cognizant that low level chronic
intoxication from cyanide is probably more widespread and is quite incapaci-
tating. The acute toxic effects of cyanide are well described (Gettler and
St. George, 1934). The problems involved in chronic exposure to cyanide under
occupational conditions (El Ghawabi, 1975 and Osuntokun, 1970) and the role of
cyanide in producing these human neuropathies (Lessell and Kauwabara, 1974).

I. CYANIDE ANTAGONISTS

Many of the present cyanide antagonists are quite efficacious. Newer cyanide antidotes are frequently reported; however, their protection is relatively small compared to some of the very effective antidotal combinations presently available. These antagonists exert their protective effects by metabolizing cyanide, binding it or by a mechanism which is still not clearly understood.

a. Metabolism of Cyanide

A thiosulfate sulfurtransferase was reported to rapidly convert cyanide to thiocyanate in the presence of the substrate sodium thiosulfate (Lang, 1933 a,b). Subsequently, sodium thiosulfate was employed to treat the lethal effects of cyanide (Chen et al., 1933 and Hug, 1933). The application of sodium thiosulfate was well conceived as this enzyme rhodanese, thiosulfate sulfurtransferase has a wide distribution biologic tissues (Himwich and Saunders, 1948), and this enzyme has a high turnover number and is essentially irreversible (Sorbo, 1953 a,b). There is a limitation with regard to the use of sodium thiosulfate as an antidote, as it does not paralle' 'he distribution of hydrogen cyanide. Moreover, the enzyme which detoxifies cyanide is present in the mitochondria in most mammals and man, and there is a limited distribution of sodium thiosulfate to these sites. Sorbo's Laboratory has attempted to circumvent the limited distribution of sodium thiosulfate by administering crystalline rhodanese in combination with sodium thiosulfate intravenously. This novel approach represents the first attempt to employ an antidote directly to antagonize the toxic effects of a chemical. The substrate specificity of rhodanese has been examined (Sorbo. 1953 b) and a few of these chemicals have been employed rather successfully as a cyanide antagonist (Clemedson et al., 1955).

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b. Binding Cyanide

1) Methemoglobin Generators

Nitrites will oxidize hemoglobin to methemoglobin and this latter chemical combines with cyanide to form cyanmethemoglobin. This latter chemical does not have a higher affinity for cyanide than cytochrome oxidase, but there is potentially a much higher content of methemoglobin than cytochrome oxidase. This would mean that the effecting list of methemoglobin is due to its mass action effect. Dr. K.K. Chen and co-workers (1933) incorporated sodium nitrite into the antidotal combination to antagonize cyanide poisoning and demonstrated that this agent can protect against several lethal doses of cyanide.

c. Cobalt Compounds

Cobalt containing compounds were the first agents used to antagonize lethal affects of cyanide (Antal, 1894). In spite of these reports that cobalt salts were effective as a cyanide antagonist, they were not widely employed because the cobalt salts were believed to be too toxic. Interest was renewed in the use of cobalt in treating cyanide poisoning when Mushett and co-workers (1952) subsequently demonstrated over a half a century later that hydroxocobalamine were useful in antagonizing cyanide. Subsequent to this report various other cobalt containing compounds were employed as effective cyanide antagonists (Evans, 1964; Estler,1966; Schwarzkopf and Friedberg, 1971; Burrows and Way,1979) and ultimately cobalt EDTA was employed in cyanide poisoning under laboratory and clinical situations (Paulet, 1958; Mercker and Bastian, 1959; Bartelheimer et al., 1962 and Nagler et al., 1978). The application of cobalt EDTA as a cyanide antagonist is attributed to its ability to form a stable complex with cyanide.

Moreover, by administering cobalt EDTA it is hoped that many of the toxic effects of cobalt could be minimized. Selection of cobalt EDTA is based on a sound pharmacologic basis as the reaction of cobalt with cyanide is rapid compared to the delayed onset of action of sodium nitrite, as there is a delay when a methemoglobin generating compound is employed.

d. Other Chemicals which Generate Methemoglobin

Various laboratories (Paulet et al., 1960 and Weber et al., 1962) indicated the possible limitations in the use of nitrites in cyanide poisoning because of the relatively slow rate of methemoglobin formation. This prompted the search for agents which were more rapid methemoglobin formers. The efficacy of amyl nitrite as a methemoglobin former was raised (Jandorf and Bodansky, 1946) and it is really doubtful that amyl nitrite would form substantial methemoglobin under the conditions that it would normally be used. Various more rapid methemoglobin formers were investigated and lead to the development of p-aminopropiophenone (Jandorf and Bodansky, 1946). A series of compounds subsequently were developed employing this as the chemical nucleus and ultimately 4-dimethyaminothenol was uncovered. The development of a more rapid methemoglobin former as a reasonable pharmacologic basis, as it has been reported in invitro studies that methemoglobin can reactivate cyanide inhibited cytochrome oxidase.

e. Cyanohydrin Formation

Whereas some laboratories were investigating the binding of cyanide with inorganic chemicals to form stable complexes, other groups directed their studies against organic chemicals which would bind directly with cyanide. Cyanide is a sufficiently potent nucleophile that it would interact with various carbonyl groups to form cyanohydrins. Sodium pyruvate

(Cittadini et al., 1971 and 1972) reported the use of sodium pyruvate to antagonize the lethal effects of cyanide. This minimal protective effect of sodium pyruvate alone was confirmed by Schwartz et al. (1979) and subsequently it was demonstrated that sodium pyruvate can potentiate the antidotal effect of sodium thiosulfate. This latter antidotal combination is not as effective as the classic combination of sodium nitrite and sodium thiosulfate; however, it's addition to this combination gave enhanced protective effect . The advantages of sodium pyruvate over sodium nitrite is that it can react directly with cyanide rather than being dependent on a secondary formation of methemoglobin. Moreover, pyruvate is more apt to bind cyanide, as it can not distribute sites of cyanide localization since it is actively transported.

II. OTHER ANTAGONISTS

a. Oxygen

In cyanide intoxication oxygen transport and oxygen tension are usually adequate and only the tissue utilization of oxygen is inhibited. Therefore, from this biochemical viewpoint oxygen has no basis in antagonizing the lethal effects of cyanide. However, it has been reported that oxygen is useful not as an adjunct but as an integral part of the treatment of cyanide poisoning (Sheehy and Way, 1968) . This effect of oxygen was demonstrated prophylactically and therapeutically (Sheehy and Way, 1968). Maximal synergism is obtained when oxygen is administered in combination with sodium nitrite and sodium thiosulfate. The pharmacological basis for this potentiation is still not clear.

III. TREATMENT OF CYANIDE POISONING

There are numerous effective agents which can be employed to antago-
nize cyanide poisoning. Therefore, there is no unanimity with regard to
the antidotal combination to be employed in treating cyanide poisoning.
Because there are numerous efficacious antidotal combinations to treat
cyanide intoxication, a discussion of the general supportive treatment
is frequently ignored. The signs and symptoms of cyanide intoxication
are described in great detail in various publications and textbooks; how-
ever, it is frequently difficult to diagnose cyanide poisoning in the absence
of a suitable history. There are very few clinical reports on acute cyanide
toxication primarily because of its rapid lethal effects. The importance
of general supportive treatment is borne out in the report of Gram and
co-workers (1977) where there was a case report on the ingestion of potas-
sium cyanide and the only treatment employed was supportive, as a diagnosis
of cyanide intoxication was not established. The dose of potassium
cyanide ingested was established at 600 milligrams. The importance of
this report is to point out that the general supportive treatment should
not be overlooked in spite of the efficacious antidotes which are available.

b. Nitrite-Thiosulfate-Oxygen

The first trial of sodium thiosulfate with the nitrites in the treat-
ment of cyanide poisoning was reported approximately fifty years ago
(Chen et al.,1933) and it is still one of the most efficacious antidotal
combinations to treat cyanide poisoning. This can be attributed to the
fact that this antidotal combination is based on a rational pharmacological
basis employing a duel mechanism of action involving the binding and

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metabolism of cyanide. This antidotal combination was based on earlier studies which indicated that cyanide either bound as cyanmethemoglobin (Bernard, 1932) or metabolized to thiocyanate (Lang, 1933)which was much less toxic than free cyanide.

It should be emphasized that the antidotal combination of sodium nitrite and sodium thiosulfate are not without their toxic affects. Precautions must be taken when this antidotal combination is administered to children (Berlin, 1970). Children under 25 killigrams should have the dose of sodium nitrite adjusted, as the adult dose recommended can be potentially lethal for children.

Concerning the advisability of using oxygen with the antidotal combination of sodium nitrite and sodium thiosulfate, since there appears to be no hazard in using oxygen and the procedure could be lifesaving, it's adoption as a routine measure appears warranted.

c. Cobalt

Although cobalt salts are one of the earliest antidotes employed to antagonize cyanide intoxication, these compounds never received general acceptance because of their toxicity until hydroxocobalamine and cobalt-EDTA were introduced. Presently,there are various proponents for the use of cobalt EDTA in the treatment of cyanide poisoning (Paulet, 1960). Whether these compounds have appreciably lower toxicity on a molar basis is still a question which is raised. More recent studies (Nagler et al., 1978; Hilman et al., 1974; Naughton, 1974) appear to relate the ventricular arrhythmias to cobalt EDTA. Although many studies indicate that sodium thiosulfate greatly enhances the antidotal effects of cobalt (Friedberg, 1968; Isom and Way, 1973) cobalt EDTA in most proprietary

mixtures do not contain any sodium thiosulfate. Various laboratories have inferred that a cobalt chelate or an aminophenol derivative in combination with sodium thiosulfate might replace the classic antidotal combination of sodium thiosulfate and sodium nitrite. Objections to the nitrite-thiosulfate combination is attributed to their slow onset of action and relatively slow detoxification (Friedberg, 1968). These conclusions were based on the rate of methemoglobin formation (Kiese and Weger, 1969) and measurements of the physiologic effects of cyanide and/or rate of excretion of cyanide. There have been questions raised whether these measurements are reliable parameters in determining therapeutic efficacy against the lethal affects of cyanide (Burrows and Way, 1973).

Although the use of cobalt complexes may be lifesaving in treating cyanide intoxication, a careful study of the toxicity of cobalt itself is essential. It's use under clinical conditions are manifesting signs of cobalt toxicity, particularly with reference to ventricular arrhthmia .

IV. ASSESSING THE TREATMENT OF CYANIDE INTOXICATION

There are numerous effective agents to treat cyanide poisoning; therefore, there are different opinions expressed with regard to the antidote or antidotal combination. Sodium nitrite and sodium thiosulfate have been used for almost fifty years and the only improvement to this combination is the addition of oxygen. A very important precaution raised with regard to the use of this antidotal combination is the toxicity of nitrite (Berlin, 1970). Moreover, questions have been raised with regard to their onset of action, particularly sodium nitrite (Friedberg, 1968; Kiese and Weger , 1969). More rapid methemoglobin formers have been developed and have prompted some reports indicating that sodium nitrite should be

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replaced with 4-dimethyaminothenol (Kiese and Weger,1969). Concerning

the use of cobalt to bind cyanide, its proponents have concentrated

predominately on cobalt EDTA (Paulet, 1960). Cobalt EDTA is used rather

widely and the main advantage is a rapid direct action to form a stable

cyanide complex, while other antidotes which bind cyanides are dependent on the

generation of methemoglobin. Cobalt EDTA would probably be more effective

if used in combination with sodium thiosulfate. However, recent reports

have raised some concern with regard to the toxicity of cobalt EDTA .

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